SEED QUALITY AND STORAGE PERFORMANCE IN MUNGBEAN AND PEANUT

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Uraiwan Supradith

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ABSTRACT

SEED QUALITY AND STORAGE PERFORMANCE IN MUNGBEAN AND PEANUT

Five seedlots of mungbean and three seedlots of peanut were assessed for seed quality using six standard laboratory tests *ie.* purity analysis, seed moisture content, germination, seed health, and two vigour tests (accelerated ageing, and conductivity (electrolyte leakage)). These testing methods were valuable as the results allowed distinction of quality differences between seedlots which were used to explain the possible cause or causes of poor quality in each seedlot, eg. high seed moisture content, low viability or vigour, mechanical damage, or fungal infection. The three highest quality seedlots of mungbean (lot 1 cv. Chinese, lot 2 cv. Berken, and lot 3 cv. Regur) and one seedlot of peanut (cv. Spanish White) were identified (germinations 88, 94, 94 and 72 percent before, and 55, 51, 66 and 67 percent after accelerated ageing), and selected to use in a subsequent seed storage experiment. Seeds were stored under different conditions involving two seed moisture contents (8.6% and 13.4% for mungbean, and 6.6% and 11.5% for peanut), two storage containers (in aluminium foil packets representing sealed storage, and muslin cloth bags representing open storage) and various temperature/ relative humidity regimes (30°C/95%RH and 20°C/75%RH for mungbean, and 30°C/50%RH, 20°C/75%RH, 5°C/85%RH , and 30°C/95%RH (open storage only) for peanut). Effect of initial seed moisture content or relative humidity, packaging and temperature on seed moisture content, germination percentage, conductivity leachate and seed health of each lot was studied at two monthly intervals during an up to eight months storage period.

In all cases, deteriorative changes were higher in open storage at high relative humidity (95%) at 30°C than at lower level relative humidity and temperature regimes. At 30°C/95%RH, seed moisture content of both mungbean and peanut seed open stored initially at low and high moisture content increased markedly to equilibrium with the

prevailing relative humidity (15-18.4%SMC in mungbean and 12.4-12.7%SMC in peanut at 2 months storage). Under these conditions all seed all seedlots lost germination after one month (peanut) or six months (mungbean) and loss of electrolytes from seeds into steep water also increased markedly with increasing storage time. Levels of infection by field fungi decreased rapidly with a concomitant rapid increase in invasion of storage fungi, such as *Aspergillus glaucus*, *A. flavus*, *A. candidus*, *A. ochraceus* *A. niger* and *Penicillium spp.*

Open stored dry and wet seedlots at lower temperatures/relative humidities of 20°C/75%RH for mungbean, and 30°C/50%RH, 20°C/75%RH, or 5°C/85%RH for peanut, reached equilibrium moisture contents oft 11.3-12.7%, 3.8, 6.5, and 7.2% after 8 months storage, respectively. Mungbean seed germination and vigour was maintained appreciably for 8 months, while peanut seed stored at an initially high moisture content showed a marked decrease in quality, particularly at 30°C. Fungal infection was generally low.

Throughout the storage period seed moisture content in sealed storage at all temperatures did not change from initial levels (8.6% or 13.4% in mungbean and 6.6% or 11.5% in peanut). Initial seed moisture content greatly affected seed germination, conductivity leachate and fungal infection, particularly in peanut seeds. Loss of peanut seed germination and seed vigour both increased with increasing seed moisture content and storage temperature. Peanut seeds stored at a higher initial level (11.5%SMC) lost all germination after 2 months storage at 30°C, after 6 months at 20°C and retained near initial levels of germination after 8 months at 5°C. In mungbean seeds stored at 13.5% SMC, seed germination and vigour were affected after 8 months storage at 30°C, particularly in poorer quality lots. The main storage fungal infection was *A glaucus* but at low levels in all cases.

Deteriorative changes were more rapid in initially poorer quality lots than in initially higher quality lots of both mungbean and peanut seed.

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CHAPTER 1
INTRODUCTION

Mungbean and peanut are important legumes which are widely grown in many countries in subtropical and tropical climates. In Thailand, these crop species are grown as a cash crop after rice and other maincrops such as maize, or cotton. Estimated planting areas of mungbean in Thailand are about 450,000 hectares (Chainuvati and Charnnarongkul, 1990) and about 118,000 hectares (Jogloy, *et al.,* 1991).

Yield and quality of these legumes is generally low due to the planting of poor quality seed. Although high quality seed is produced by the official government agencies such as the Seed Division of the Department of Agricultural Extension, the amount available is generally insufficient to meet planting requirements. Therefore many growers are forced to purchase poor quality seed for planting from local merchants which often results in low financial returns from the subsequent crop.

Poor seed quality is due mainly to seed deterioration during storage since, mungbean and peanut seeds are normally required to be stored for about 6-8 months from harvest to the next planting season. Such storage is usually under ambient conditions of high temperature (around 30°C) and relative humidity (above 85 %).

The deterioration rate of seed is caused by its inherent characters (Delouche *et al.*, 1973). In peanut seed in particularly high oil content makes it difficult to successfully prolong seed viability (Woodroof, 1973). Moreover, pre-storage history and storage conditions also influence seed storability (Delouche, *et al.* 1973; Justice and bass, 1978; Roberts, 1986) since adverse environmental conditions eg. high temperature and high relative humidity or frequent rainfall during seed development, and maturation, and at harvest result in 'seed weathering' and high seed moisture contents which lead to increased susceptibility to infection by pathogens and to bruising during mechanical harvesting. The

traditional sun drying of seed to very low moisture contents can also lead to seed cracking damage during threshing and processing, particularly where inappropriate methods, for example, high cylinder velocity mechanical threshing system are used (Justice and Bass, 1978; Heslehurst *et al.,* 1987). These factors can all contribute to poor seed viability prior to storage. a situation which continues in storage, particularly if seed is stored at high temperature and high relative humidity or high seed moisture content (Barton, 1961; Delouche *et al.*, 1973; Justice and Bass,1978). Under adverse storage environments storage fungi also play an important role in speeding up the processes of seed deterioration (Christensen, 1972, 1973; Neergaard , 1977; Agarwal and Sinclair, 1987).

Under tropical humid climates (of high temperature and relative humidity) such as that experienced in Thailand, these leguminous orthodox seeds require dry and cool conditions for viability preservation. Thus, in ambient storage conditions seeds may become non-viable in a short time and often well before the next sowing season. In some cases, seeds are stored at lower temperatures, but dehumidified cold storage is a much better solution. The compromise is often considered to be storage in an air conditioned room at 20°C, as a compromise but cheap for seed storage system (Hill,1995b). Moisture-proof packaging such as the storage of seed in aluminium foil packets can be an improved option to maintain low seed moisture content under high ambient relative humidity and to protect seeds from pest invasion (Justice and Bass, 1978; Arvier, 1983). However, it is not always appreciated that the level of seed moisture content for moisture-proof packaging needs to be considerably lower than for open storage to be effective (Hill,1995b). Safe sealed storage moisture levels vary depending on crop species, as well as storage temperature and duration. The question is "what options are most suitable for short-term storage of mungbean and peanut seed in Thailand".

Since pre-storage history remains an important factor influencing the preservation seed viability, the present study was carried out in an attempt to evaluate laboratory seed testing methods to determine initial quality of different seedlots of mungbean and peanut

and to explain the likely causes or causes of differential pre-storage quality of different seedlots. Similar work on maize has been carried out by Sakunnarak (1985), and maize and soybean by Koolkaew (1991). Understanding more about the relationships between seedlot differences, initial seed moisture content and quality, and of the packaging and storage environment on mungbean and peanut seed performance during storage will help to select appropriate storage conditions for these legumes in Thailand. Therefore, the present study aimed to :

1. determine the value of different laboratory methods for assessing pre-storage history.

2. determine the effects of different storage conditions on seed deterioration rate.

3. compare relative storability differences between mungbean and peanut seed.

4. establish recommendations which could be implemented to increase seed storage life of mungbean and peanut seed in Thailand.

CHAPTER 2
REVIEW OF LITERATURE

2.1 SEED QUALITY AND STORAGE PERFORMANCE

The term "seed quality"refers not only to analytical purity and germination capacity, but also includes species and cultivar purity, seed size, seedlot uniformity, seed moisture content, seed vigour, and seed health as components of seed quality for sowing (Thompson, 1979). The quality of fresh harvested seed can be affected by several factors during seed production and by harvesting procedures. Seed processing can improve some components of quality, eg. analytical purity, seedlot uniformity and seed moisture content, but improper procedures can damage seed, resulting in a reduction in seed quality such as the germination and vigour of seed.

Seed is normally stored for various lengths of time depending on the it's future purpose. For example, short term storage is to preserve seed quality from harvest to the next growing season (approximated 1 to 9 months), intermediate term storage is to carry-over seed stocks for 2 or 3 seasons (18-24 months) as an insurance against disasters, and long term storage is regularly used for storage of breeders seeds, requiring preservation of quality for up to 10 years (Delouche *et al.*, 1973; Arvier, 1983).

The first factor that causes differences in seed performance and storability of seed is the initial quality of the seed entering storage. Storage environmental conditions, such as temperature, moisture, hygiene and protection of seed, can affect seed quality markedly. The effects of these factors on seed quality and storability are reviewed covering orthodox crop species, particularly legume seed. The process of seed deterioration involving seed testing methods to assess and distinguish quality differences between seed and seedlots will also be reviewed.

2.1.1 FACTORS THAT AFFECT SEED QUALITY AND STORABILITY

Following physiological maturity, seed begins to deteriorate over time. The rate of deterioration during storage can be influenced by several factors such as genetic effects, seed structure and composition, maturity, weather damage, mechanical damage during harvesting or processing, pest and fungal damage, chemical treatments, and storage environmental conditions.

2..1.1.1 Genetic effects

Storability and quality of seed varies between species due to genetic effects (Justice and Bass, 1978; Delouche *et al.*, 1973). Seed of some species are inherently long-lived, and able to maintain viability under a given set of storage conditions better than others which are relatively short-lived. Seed of some legumes such as *Cassia* and *Trifolium* are able to survive for periods of more than 100 years (Harrington, 1972). However some legume seeds, such as peanut and soybean, are short-lived and are therefore classified as poor storers, while others such as *Phaseolus spp.* and maize are classified as intermediate storers (Delouche *et al.*, 1973).

Variation in the ability of seed to retain quality also differs among cultivars of the same species. For example, Toole and Toole (1954) noted that The bean seed variety 'Black Valentine' stored better than seeds of the 'Brittle wax' variety. Powell *et al.* (1986), and Taylor and Dickson (1987) also found that the black-seeded cultivar of *Phaseolus vulgaris* had a slower rate of water absorption and less damage during imbibition than the white-seeded cultivar. In soybean, Delouche (1969) noted that the seeds of modern cultivars are often inherently short-lived and more susceptible to field weathering and damage than older cultivars which had thicker seed coats. Similar associations were found in mungbean by Williams *et al.* (1995a) where seeds of black gram cv. 'Regur' showed a slower rate of water absorption and relatively more resistance to weathering damage than seeds of green

gram cv. 'Berken', due possibly to differences in the impermeability of the seed coat.

2.1.1.2 Seed structure and composition

Seed morphology differences, including shape, size and the arrangement of essential structures respond differently to mechanical damage during harvesting, handling, and processing. In general, small seed escapes mechanical damage more than large seed. While irregular or flat shaped seeds are more susceptible to mechanical damage than spherical seeds (Moore, 1972). Legume seeds such as beans and peanuts are highly sensitive to damage, and this is due to their large cotyledons and the location of the embryo axis only tolerate low level impact (Gelmond, 1962; Justice and Bass, 1978).

Chemical composition of seed is another factor that can affect seed quality and longevity. Seed normally contains carbohydrates, proteins, lipids (fats and oils) and minerals. For example, kernels of peanut contain about 25% protein, 10-15% carbohydrate, and 40-50% lipid, and these components vary with variety, seed size, season, and cultural practices (Salunkhe and Desai, 1986; Kadam *et al.*, 1989) while mungbean seed contains approximately 25-28% protein, 62-65% carbohydrate, and 1-1.5% oil (Lawn and Ahn, 1985). Seed which contains high levels of lipids, such as peanut, seem to deteriorate more rapidly than the others (Barton, 1961; Bennett-Lartey, 1991).

2.1.1.3 Seed maturity

Maturity is considered to be the stage when seed reaches its maximum dry weight. Variation in seed at this stage can affect seed quality and longevity, eg, low density, shrivelled, and small seeds in the population are generally immature or partially filled, exhibit low germination and vigour and do not store as well as mature seed (Baskin and Delouche, 1971a; Bass, 1980; Delouche, 1980; Dharmalingam and Basu, 1988; Delouche, 1992).

Mungbeans and peanuts flower indeterminantly are in various stages of maturity at harvest. It is therefore difficult to determine optimum harvest time. Seed of peanut is normally harvested when two thirds to three quarters of the seed is fully mature. At this stage seed moisture content is approximately 50% (Reusche, 1987), and therefore seedlots contain variable amounts of immature and mature seed, hence affecting the quality of the seedlot.

2.1.1.4 Damage by weathering in the field

The deterioration of seed begins from the time that seed reaches physiological maturity or attains maximum quality during development in the field (Hampton, 1994a). At this point the moisture content of most seed is too high for efficient harvest (eg. approximately 50% in soybeans, peanuts, and beans), and are usually allowed to dry-down on the plant to be harvested when harvest maturity is reached (Delouche, 1980). Alternatively, seed is allowed to dry in the windrow to a safe moisture content of around 20% or less before threshing. The environment during this period is often unfavourable for seed, causing rapid seed deterioration and lowering of seed quality (Delouche *et al*, 1973). Seed of legumes such as mungbean and peanut are very susceptible to field weathering. To minimize the degree of weather damage, selection for synchrony of maturity can reduce the period of vulnerability in the field (Park and Yang, 1978; Heslehurst *et al*., 1987), and/or avoid conditions which cause weathering by manipulating sowing time and harvesting time (Bott and Kingston, 1976).

High temperatures and relative humidity play a critical role in field weathering. High temperatures and dry weather cause rapid drying and can literally "cook" seeds, rendering them nonviable (Harrington, 1972). While high relative humidity or rainfall can delay ripening, increase respiration, and reduce the accumulation of dry matter in the cotyledons (Howell *et al*., 1959). All of these factors can contribute to reduced seed vigour. Williams *et al.* (1995b) also reported that rainfall or high humidity resulted in changes to

the morphology of mungbean seeds by increasing the proportion of seed with discoloured and/or wrinkled testas, inducing cracking of the testa and in extreme cases even inducing sprouting of seed. These problems increase with extended periods of bad weather. Similarly, high soil moisture content can cause mature peanut seed to sprout in the pods, if harvesting is delayed (Gelmond, 1962). However seed damage caused by weathering results mainly from alternating wet and dry conditions in the field. High relative humidity causes swelling and if followed by extremely dry weather can cause contraction of the seed coat and cotyledonary tissue associated with imbibition and dehydration (Austin, 1972). Unequal expansion and shrinkage of this tissue results in cracking, ruptured and brittle seed coats and produces streaks or scars on the radicle and cotyledons (Moore, 1972). High temperatures accentuate adverse moisture effects when both factors occur together. These factors result in a reduction in seed germination, and vigour, and can provide optimum conditions for the invasion and growth of microorganisms and pests in both pods and seed, particularly when harvest is delayed (Gelmond, 1962; Heslehurst *et al.*, 1987; Lassim *et al.*, 1984).

2.1.1.5 Mechanical damage

Incorrect harvesting, threshing, and processing, can cause mechanical damage to seed, whether these process are done manually or by machine. However, incorrect mechanical methods are generally the main cause of seed injury, and the loss of harvested seed due to breakage can be more than 20 % (Toole *et al.*, 1951; Wilson and McDonald, 1992).

The degree of damage is affected by:

i) seed structure including size and shape, eg. larger seed, elongated or irregularly shaped seed, and fragile seeds with brittle coats (large-seeded legumes) are more easily damaged than smaller seeds eg. grasses (Moore, 1972). These problems also vary between

species and cultivars (Dickson and Boettger, 1976), eg. peanut seed is composed of 2 cotyledons, epicotyl and radicle, surrounded by a thin testa. The radicle protrudes slightly out of the cotyledons at the base of the seed. As a result it is very susceptible to injury, which causes a reduction in germination. However damage to the end of the seed opposite the radicle does not affect germination (Gelmond, 1962).

ii) the resistance and hence the force required to remove the seed from the pod or plant (Justice and Bass, 1978).

iii) moisture content of the seed and pod, and

iv) the velocity of the machine (Justice and Bass, 1978).

Seed moisture content and the severity of machine action are the most important factors causing mechanical damage. It is therefore necessary to consider the safe moisture content of seed before harvesting and processing. Harvesting and threshing at too high a seed moisture content eg. above 20% in mungbean (Herath *et al.*, 1981), and in soybean (Prakobboon, 1982) results in seed bruising and internal breakage, which are often invisible and may not affect germination immediately, but can hasten seed deterioration in storage. This damage may relate to toughness or softness of moist seed tissues such as the seed coat and cotyledons (Moore, 1972). On the other hand, the harvesting of seed with too low a moisture content (eg. below 12% in beans and soybean) can cause cracking and splitting of the seed due to its very dry and brittle condition, with the degree of damage increasing as the seed moisture content decreases (Green *et al.*, 1966; Moore, 1972; Herath *et al.*, 1981; and Prakobboon, 1982).

The cylinder velocity of the machine during combining, threshing, and shelling can cause seed damage which will increase as the cylinder speed is increased eg. speeds above 500 rpm can cause serious damage to soybean seed (Green *et al.*, 1966). However, the severity of this damage is mostly influenced by the interaction between the level of seed moisture content and the velocity of the machine (Justice and Bass, 1978). Harrington (1972) recommended that seed damage may be decreased by reducing cylinder speed and

coating the beater bars with rubber.

Such mechanical damage results in decreased germination and vigour, and increased levels of abnormal seedlings depending on the extent and site of damage. The breakage of essential structures eg. seedlings with broken epicotyl, or cotyledons broken from the embryo axis when seed is germinated (Harrington, 1972; Sader *et al.*, 1991) are particularly damaging. Abnormal seedlings due to curling at the base of the hypocotyl may occur if the tip of the embryonic root is damaged (Gelmond, 1962). Peanut seed frequently shows radicle damage due to its protrusion beyond the cotyledons. Furthermore, seedlots showing damage from machine operations do not store as well as seedlots which are hand harvested (Baskin and Delouche, 1971*b;* Subbaraman and Selvaraj, 1989) and are easily invaded by microorganisms.

2.1.1.6 Seed damage caused by heat

High seed moisture is the greatest cause of losses in seed quality (Harrington, 1959). Therefore, freshly harvested seeds which may still contain high moisture content (around 40% in peanut after lifting from the soil) must be rapidly and safely dried to a safe moisture content level for shelling and subsequent storage. Heat, either from sunshine or from artificial drying is used to lower seed moisture content. Because seeds are thermosensitive, the damage to seed by heat can occur as a result of the cumulative effects of respiration and fungal heating and by the accumulation and retention of radiant heat within the seed mass following harvest ('field heating'). Similar damage due to high temperatures can also occur due to excessive heated air in artificial drying systems, commonly called 'drying damage' (Hill, 1995b). Drying moist seed with too high a temperature will accelerate the rate of water evaporation from the seed surface into the air. If the speed of water evaporation from the seed surface is faster than the rate of water diffusion from the central parts of seed to the surface, this will cause a 'break' in the water vapour gradient and may result in the inside of the seed remaining wet after drying. In storage, this damaged seed will become 'sticky',

exhibit high respiration, and provide favourable conditions for the growth of microorganisms that increase heat accumulation in the seed mass. This is reflected in lower seed quality. The deterioration will show in a few weeks as a lowered interim germination count, followed by an increase in abnormal seedling percentage which shows as seedlings with broken parts, restricted roots without root hairs, stunted plumules and ultimately seed death (Mackey, 1972; Hill, 1995b). Also, drying wet seed with too high a temperature can cause heat accumulation in the seed bulk and cause cracking and splitting in the seed coat, cotyledons or embryonic axis leading to increases in abnormal seedlings and dead seeds.

2.1.1.7 Seed damage caused by fungi

Many kinds of pathogens such as fungi, bacteria, viruses and nematodes can cause seed losses in terms of yield and quality in the field and in storage. Fungi play an important role in determining the quality and longevity of seeds. Some are serious parasites of mungbean and peanut seeds, while others are saprophytes or very weak parasites. From an ecological standpoint the fungi that invade seeds can be divided into two general groups, viz. field fungi and storage fungi (Christensen ,1973).

Field fungi

Field fungi are those that contaminate or infect seeds on developing plants in the field, or during ripening, or during harvest and awaiting threshing (Christensen, 1973). These fungi can cause diseases which mostly result in a reduction in seed yield. Some diseases also affect seed quality and can be seed-borne or transmitted through seeds. Field fungi can cause damage to seeds either singly or in combination with the following disorders: seed abortion, shrunken seed, seed rot, seed necrosis, sclerotisation, seed discolouration, reduction of germination percentage, increased levels of decayed abnormal seedlings, and physiological changes in seed (Neergaard, 1977).

There are several genera of field fungi that invade mungbean and peanut seed. The number of seed-borne fungi generally increases when harvesting is delayed. The following are examples of seed-borne fungi and their symptoms found on mungbean and/or peanut seeds. *Macrophomina phaseoli* causes seed coat discoloration by producing necrotic black, brown to grey spot and concealed damage in peanut (Williams and McDonald, 1983 and Porter *et al.*, 1984). *Alternaria spp.* causes seed discoloration by producing necrotic lesions and produces both mycelium and sporulating structures on seeds. *Fusarium spp.* causes seed discoloration similar to *Alternaria spp.* and is important in causes damping-off and seedling rot. *Colletotrichum spp.*, which penetrates into the fleshy cotyledons, produces conspicuous necrotic lesions in beans (Hall, 1991). *Rhizoctonia solani* causes seed decay and damping-off of seedlings; hypocotyl and root necrosis and root rot. *Pythium spp.* causes pod and seed rot in peanut, and the seedlings that emerge are stunted, with root rot, and leaves generally light green and often necrotic (Porter *et al.*, 1984). *Sclerotinia spp.* infect peanut seed causing seed discolouration and seed rot. *Sclerotium rolfsii* causes seed rot and bluish discolouration on the testa of infected peanut seed due to the production of oxalic acid and phytotoxins produced by the fungus (Williams and McDonald, 1983 and Porter *et al.*, 1984).

In general, field fungi require a seed moisture content in equilibrium with high relative humidity of more than 90 percent for their growth, and as stated, are generally confined to the field. In storage where seeds are stored with moisture contents below those required for field fungi growth, field fungi infection decreases with increasing storage time, but the fungi may alive for years in seeds stored at low moisture content. Their survival favour low moisture content and low temperature (Christensen and Kaufmann, 1965). However, if seed, taken from the field is stored at high moisture content or high relative humidity, awaiting processing, then conditions will allow some so-called field fungi (eg. *Fusarium* and *Alternaria*) to develop and cause rapid seed deterioration.

Storage fungi

Storage fungi are those that grow on seeds in storage and are the major cause of seed deterioration during storage. They comprise 10-15 species of *Aspergillus*, and several species of *Penicillium* (Christensen and Kaufmann, 1974). Christensen (1973) stated that these fungi do not normally invade seeds to any serious extent before harvest. However, Porter *et al.* (1984) reported that peanut seeds can be often infected by *Aspergillus niger* in the field which causes crown rot, and *Aspergillus flavus,* which causes yellow mold and aflatoxin contamination particularly in over-mature and damaged seed. These fungi also contaminate overmature mungbean seed in the field (Teggi and Hiremath, 1990; Thakur *et al.*,1990). Both, however, can only become important in storage and extremely damage to seed stored under unfavourable conditions, such as hot and wet environment.

Generally, storage fungi have the ability to grow in seeds with moisture contents in equilibrium with relative humidities of 65-70% where no free water is available (Christensen, 1973; Neergaard, 1977). The invasion of *Aspergillus spp.* increases as the relative humidity of the storage environment increases. They also often tolerate high temperature, and hence are grouped as 'Thermophilic' (Hill, 1995a).

Invasion of stored seed by storage fungi can result in a reduction of seed germinability, cause seed discolouration, heating and mustiness, production of mycotoxins such as aflatoxin, spoilage in nutritive value, seed caking and decay in the final stages of seed deterioration and loss in weight (Neergaard, 1977; Agarwal and Sinclair, 1987). In addition, a range of other organisms, such as bacteria, may grow and accelerate the process of seed deterioration. The following are the common storage fungi invading seeds in storage.

Aspergillus restrictus can kill and discolour germs, the minimum relative humidity for its growth at temperatures around 27-30°C is about 70%, or at a range of seed moisture contents varying between seed species eg. 12-12.5% in soybean or 8.5-9.0% for sunflower, safflower and peanuts (Christensen and Sauer, 1982). The fungus is likely to

result in germ damage and mustiness in grains stored for several months to a year. Since it grows very slowly, it does not cause heating (Christensen, 1972).

Aspergillus glaucus can kill and discolour germs at a range of seed moisture contents around 9.0-9.5% in peanuts (Christensen and Sauer, 1982), or 12.5-13% SMC in soybean or at a relative humidity of 73% at temperature around 27-30°C. The rate of fungal activity is very slow at lower moisture contents and more rapid at higher moisture contents. It also causes mustiness, caking, and can increase seed temperature to 40-45°C ,then gradually increases seed moisture content thereby providing favourable conditions for *A. candidus* to grow rapidly. Heating and spoilage then follows within a few days (Christensen, 1972).

Aspergillus candidus can kill, discolour germs of seeds and whole kernel rapidly, and then cause total decay. The lower limit of moisture for growth is about 9.0-9.5% in peanuts (Christensen and Sauer, 1982), or 14.5-15%SMC in seeds of soybean, or at a relative humidity of 80% at 27-30°C. It also causes heating up to 55°C and spoilage within a few days to a few weeks (Christensen, 1972)

. *Aspergillus ochraceus* prefers the same growth conditions as *A. candidus*. It can kill and discolour germs, and produces a toxin (ochratoxin) (Christensen, 1972, Christensen and Sauer, 1982).

Aspergillus niger causes seed rot and pre-emergence damping-off that results in decayed abnormal seedling when germinated (Porter *et al.*, 1984) . The fungus can grow and damage to embryo and kernel when seeds were stored at above 90 % relative humidity (Semenniuk, 1954). *A. Niger* is a poor competitor which favours high temperature and can not persist storage at low seed moisture content (eg. 6.5 % in soybean) (Agarwal and Sinclair, 1987).

Aspergillus flavus can kill, or discolour germs and whole kernels, and cause total kernel decay when seeds are stored at a relative humidity of 85% at 27-30°C, or seed moisture content of 17-17.5% in soybean or 10.0-10.5% in peanuts. It also causes rapid heating up to 55°C and produces aflatoxin (Christensen, 1972; Christensen and Sauer, 1982).

Penicillium spp. can grow in seed at moisture contents of around 10-15% in peanuts (Christensen and Sauer, 1982) or 16-18.5% in soybean or at relative humidity of 80-90% at 27-30°C which some species also active at 5°C in wet seed. It can kill and discolour germs and whole kernels or seeds, causes mustiness and caking, may be involved in the early stages of heating although it is not implicated in the rapid heating associated with infection by *A. candidus* and *A. flavus*. It also produces toxic compounds (Christensen, 1972).

Such deleterious effects of *Aspergillus spp.* on seed deterioration depends upon of species of *Aspergillus* and their capability to growth at a given storage environment, for example pea seeds stored at 85% relative humidity and 30°C were killed in 3 months when inoculated with *A. flavus,* while in 6 months with *A. candidus* and *A. ruber* and in 8 months with *A. restrictus* (Agarwal and Sinclair, 1987). The growth rate and survival of storage fungi in seeds may not be only affected by seed moisture content or relative humidity, but also temperature, $O_2/C0_2$ levels, the length of time seed is in storage and the amount of damaged seed and foreign material present. The minimum, optimum, and maximum temperature for the growth of most storage fungi is about 0-5°C, 30-33°C, and 50-55°C, at low temperature (below 10°C) the fungi grow very slowly even at above 85% ralative humidity and most storage fungi do not develop below 0°C, but a few species of *Penicillium* can grow slowly at 0-5°C and around 85% relative humidity (Christensen, 1973; Agarwal and Sinclair, 1987).

Under strict anaerobic conditions, some storage fungi may grow to some degree,

ranging from 20-50%, as much as that under aerobic conditions and acid-forming bacteria may predominate, dueing to their ability to grow under low oxygen and high carbon dioxide conditions (Christensen, 1972).

2.1.1.8 Seed damage caused by pests

Seed can be damaged by pests during its development in the field and when stored in the warehouse. In the field, there are many types of pests capable of attacking immature and mature seeds. The most serious are pod-sucking bugs (*Nazara viridura*) may pierce seeds through the pod walls, caterpillars of the moth *Heliothis spp.* may chew holes through the pod walls, and beetles (*Callosobruchus spp.*) may bore through and feed on mungbean seeds (Lawn and Ahn, 1985). This damages the embryo and results in weak seedlings or seedlings with missing structures and leads to seed death. While larval of corn rootworm and wireworms can bore into immature and mature pods of peanuts to feed developing seed (Porter, *et al.*, 1984), and also white grubs and termites can damage peanut seeds in the soils (Neergaard, 1977).

In storage, beetles, moths and mites are important storage pests which attack and feed on seeds particular under storage conditions in tropical or subtropical environments *i.e.* at temperatures of 20°C-30°C or greater. *Callosobruchus maculatus* (pulse beetle, bruchid, weevil) and *C. chinensis* are the most destructive Bruchid species in mungbean. *Oryzaephilus spp.* (sawtoothed grain beetles), *Tribolium castaneum* (rust red flour beetle) and *Ephestia elutella* (moth) can damage peanut seed. In addition insects cause damage to the seed by feeding on the seed, and their activity can cause heating, increase a moisture content and carbon dioxide in seedlots and thereby promote mould invasion, reduce seed vigour and germination, and finally cause seed death (Howe, 1973; Neergaard, 1977).

Factors which dispose seed to insect attack in store are the presence of dust and broken seeds, a high seed moisture content, high temperature, poorly designed warehouse

construction and the use of unclean containers (Parkin, 1963). Hence, the avoidance of these factors can be used as an addition protection against seed deterioration.

2.1.1.9 Chemical treatment damage

The application of chemical seed treatments, such as insecticides and fungicides particularly if excessive, may induce deformed abnormal seedlings with stubby, thickened roots and/or weak plumules and ultimately lead to seed death. For example, Nijenstein and Ester (1990) found that abnormal seedlings of field bean, with stunted stubby primary root and/or no secondary roots at the time of evaluation, resulted from an overdose of insecticide application eg. carbofuran 19.1 ml. per 1 kg seed. Injury due to chemical overtreatment of the seed in peanut has also been noted by Gelmond (1962). Moore (1963) and Neergaard (1977) reported that the deleterious effects of mercuric fungicides, such as ethylmercuric chloride, can be traced to seed injury and high seed moisture content. Superphosphate and 2-4 dichlorophenoxyacetic acid can also cause thickened and shortened seedling hypocotyls and stunted or stubbed roots in soybean (Koolkaew, 1991).

2.1.1.10 Storage environmental conditions

Seed moisture content and the storage environment (temperature, relative humidity, gases etc.) can have major effects on the maintenance of seed quality during storage. Of these, temperature and especially seed moisture content are the most crucial factors (Delouche *et al.*, 1973; Justice and Bass, 1978).

Seed moisture content and relative humidity.

Since seeds are hygroscopic, seed moisture content is controlled by the relative humidity of the surrounding air. Seed can absorb moisture from a moist ambient atmosphere resulting in its moisture content gradually increasing to equilibrium. In contrast

seed can lose moisture in a dry ambient atmosphere until its moisture content equilibrates with relative humidity of the storage air. Another important fact that must be considered is that at a given relative humidity, equilibrium seed moisture content decreases slightly with increasing temperature. The equilibrium moisture content of seed varies with species, and also depends upon the relative humidity and temperature of the ambient air, and the hysteresis effect (Delouche *et al.*, 1973; Justice and Bass, 1978). The 'Hysteresis effect', is defined as the difference reflected in the rate of absorption of water by a dry seed compared with the rate of water desorption or loss by a wet seed. The equilibrium moisture content of the latter will be slightly higher than the former (Roberts, 1972; Justice and Bass, 1978).

Factors that affect the rate of water absorption and retention by seeds include the thickness, structure, and chemical composition of the seedcoat. Of the various seed constituents, proteins are most hygroscopic, carbohydrates are slightly less, and lipids are hydrophobic (lacking an affinity for water). Therefore, at the same temperature and relative humidity, oily seeds, such as peanuts, will have an equilibrium moisture content which is lower than starchy and protein seeds. For example, at 25°C and 75% RH peanut seeds have an equilibrium moisture content of about 9.8% (or between 8.5-11.1%) while soybean seeds which are rich in protein, have a moisture content about 13.1% (or between 11.5-14.8%) (Justice and Bass, 1978). There are no differences between dead seed and live seed in terms of moisture uptake and retention.

The level of seed moisture content can affect both the quality and storability of seed (Delouche *et al.*, 1973). Harrington (1959) has established a "rule of thumb" providing guidance on the effect of seed moisture content on the storage life of seed. He stated that "each 1 percent increase in seed moisture content halves seed storage life ". He later reported that this relationship is correct for many kinds of seed with in the moisture content range of 5-14% (Harrington, 1972).

High relative humidity and consequently moisture content of seeds is the most

important cause of seed deterioration or losses in viability, germination, and vigour during storage. Singh and Yadav (1987) found that mungbean seeds with an initial moisture content of 16%, showed a decrease in germination to 1% after 6 months storage in airtight conditions at 27°C while seeds with a moisture content of 12%, showed no change in germination after two months storage but afterwards decreased. Similar studies have also reported that mungbean seeds can be stored for 3 months at 26°C without a significant reduction in germination or damage from insects or fungus if dried to a moisture content of 8-12% and stored in sealed containers (Chin *et al*, 1978). Luan (1976) also reported that peanut, when stored with an initial moisture content of 9.3% in a sealed container under ambient conditions in the tropics, showed a rapid decrease in germination percentage and most seeds were dead after 20 weeks storage. However similar seeds stored with 6.17% moisture content retained their germination above 80% during the same storage period.

Another effect of seed moisture content level on seed quality and storability is the drying seed to very low moisture contents prior to storage which may cause desiccation injury (Justice and Bass, 1978). Although seeds of some crop species can be dried to 5% and some dried to 2 to 3 percent moisture content (eg. peanut seed) without significant desiccation injury (Justice and Bass, 1978; Roberts, 1983; Ellis *et al.*, 1990), they recommended that seeds should not be dried below 3-4 percent moisture content because extremely dry seeds may be damaged by too rapid rehydration when planted (Justice and Bass, 1978). Symptoms of desiccation injury may appear after storage as cracked cotyledons, damage to the food transport system in the embryo, stunted radicle with heavy development of secondary roots, stubby primary root and shoot, protrusion of the radicle without further development, and decreased germination and vigour.

The role and effect of moisture content on seed storage has been summarised by Harrington as follows (Harrington, 1972; Neergaard, 1977).

range of seed moisture content(%)	situation in storage
above 45%	germination occurs
18-20% or above	heating may occurs
12-18% or above	mould grows on or in seeds
8-9% or below	little or no insect activity
4-8% or below	safe for sealed storage.

Temperature

Temperature is the second most important environmental factor affecting the deterioration rate of seed in storage. Harrington (1959) proposed a helpful "rule-of-thumb" guideline to indicate the influence of temperature on seed storage life i.e. for each 5°C decrease in seed storage temperature seed storage life is doubled, within the range 0 to 45°C.

In general, seed quality is reduced as time of exposure to high temperatures is increased, and as seed moisture content is increased. Delouche *et al.* (1973) also noted that seed moisture content and temperature reinforce and compensate each other in their effect on seed storage. At a given temperature, seed damage is diminished as moisture content of seed is decreased (Justice and Bass, 1978). Most field crop seeds with high moisture content of about 14-16% can be stored for a year or more at 10 °C or lower, while low moisture seed (10% or less) can be stored at 30-34°C for the same period without appreciable loss of viability (Delouche, 1968).

Packaging

The "package" used to store seed is generally made of cotton, jute, paper, plastic, metal or various combinations of materials eg. aluminium foil laminated to polyethylene and paper. Each material has characteristics that suit a particular type of package or use.

Thus, when choosing a satisfactory package it is necessary to consider which provides the most convenient and effective unit for handling, transport and storage. It must also protect the seed from contamination and mechanical damage, from the influence of climate and hazards incurred during transport, from losses or deterioration of seed during storage (Thomson, 1979; Warham, 1986).

Particularly when seed is stored under humid tropical conditions where relative humidity and temperatures are generally high, moisture proof or moisture resistant packages recommended for use must protect the seed against the damaging effects of high relative humidity on seed viability and longevity. Polyethylene film is the most common material which is resistant to water vapour transmission and is often used as an inside liner to a cloth or multiwall paper bag (Hill, 1995b). This material allows transmission of water vapour in varying degrees, depending on its thickness and density. Under tropical conditions, 175 micron, high density polyethylene or 250 micron, regular polyethylene has proved satisfactory for seed packaging. Aluminium foil pouches laminated to polyethylene, cellophane, Mylar or paper are virtually moisture proof when heat sealed and have proved to be successful water barriers equal to metal containers which are reported to be the best form of packaging (Hill, 1995b). These types of containers can maintain viability of seed better than paper bag and cloth bags through which moisture migration may occur. However, seed must be dried to safe moisture levels prior to storage in impermeable containers. Peanut seed can be stored safely at 6% moisture content or up to 9% if the temperature of the storage room is low and period of storage short (Barton, 1961; Gelmond, 1971; Norden, 1981). However seed moisture content should not be above 9% because respiration of the seeds will increase and create favourable conditions for growth of microorganisms (Harrington, 1973a, 1973b).

2.1.2 Processes of seed deterioration

Losses of viability of seed with time and ultimately seed death are the result of seed

deterioration or seed ageing. The mechanisms leading to this deterioration of seed are not well understood, Although it is known that there are many biological, physiological and cytological mechanisms involved in this process. The symptoms of seed deterioration may appear as damage to cell membranes, genetic changes, changes in respiration activity, impaired protein and RNA synthesis, changes in enzyme activity, accumulation of toxic metabolites and microbial activity (Priestley and Leopold, 1979, Pearce and Abdel Samad, 1980; Roberts, 1986, Wilson and Mcdonald, 1986; Powell, 1988; Coolbear,1994). These lead to an increase in abnormal seedlings, reduced germination and vigour of seed, and finally the seed dies. It is very difficult to distinguish which are the primary causes of seed deterioration and which are the secondary effects. However, it is believed that damage to membranes and damage to the genome must be the prime causes for loss of seed quality in ageing (Coolbear, 1994).

Membrane damage

Seed deterioration, either under natural or accelerated ageing at high temperature and moisture contents, increases membrane permeability, which leads to greater loss of electrolytes, by leakage during imbibition and results in a reduction in germination (Priesley and Leopold, 1979; Pearce and Abdel Samad, 1980; Wilson and McDonald, 1986; Powell, 1988; Perez and Arguello, 1995). Damage to membranes may be due to lipid peroxidation following free radical production which is often found to be correlated with a decline in viability and vigour. Thus, free radical damage to membranes may be the main cause of seed deterioration. Lipid peroxidation by free radicals may make membranes susceptible to hydrolytic attack by enzymes such as proteases and phospholipases (Villiers,1973).

In addition, lipid peroxidation leads to loss of membrane permeability and integrity, loss of phospholipid from membranes and loss of ultrastructure.

Loss of lipids through the membrane, particularly phospholipids, is often associated

with seed deterioration during ageing (Priestley and Leopold, 1979; Pearce and Abdel Samad, 1980; Powell and Matthews, 1981). However, Priestley and Leopold (1983) found little change in phospholipid levels in naturally aged seeds of soybean. This result was different from mechanisms of artificial accelerated ageing where there were marked losses, especially in phosphatidyl choline (Priestley and Leopold, 1979), which suggests that it was caused by hydrolysis in the early stages of the ageing process. Thus, differences in this aspect of deterioration may be influenced by moisture status of the seed during ageing and by the variety used.

During long term dry storage of seed, lipids may be slowly attacked by oxygen, forming hydroperoxides, other oxygenated fatty acids and free radicals. The free radicals are unstable and may react with and damage nearby molecules. The seed is too dry to repair this damage which accumulates during storage, thus increases in oxygenated fatty acid may be an event of deteriorated seed. These oxygenated fatty acids may be broken down by hydroperoxide lylase when seed imbibes water which leads to increasing free radicals and the production of toxic substances that can inhibit respiration, protein synthesis, DNA synthesis, and denature protein (Wilson and McDonald, 1986).

Many researchers have reported that peroxidation is associated with reduced polyunsaturated fatty acids and with loss of viability (Powell, 1988). In peanut seeds, storage lipids might be important because of their large quantities, and because seeds contain 47-50% of the lipid as oleic (45-50%) and linoleic (25-30%) fatty acids (Woodroof, 1973; and Kadam *et al.*, 1989). These are unsaturated fatty acids which are likely candidates for lipid peroxidation (Wilson and McDonald, 1986). Lipid peroxidation reactions cause changes in unsaturated fatty acid levels, influence membrane integrity and lead to increased leakage of metabolites in aged seeds of peanut (Nautiyal and Zala, 1991). The embryonic axis appears to be the part most sensitive to deterioration (Perez and Arguello, 1995).

Ultrastructural changes in seed tissues including coalescence of lipid bodies (Anderson *et al.*, 1970) withdrawal of plasmalemma from the cell wall, distension of the outer mitochondrial membrane (Hallam *et al.*, 1973) and nucleus damage are evidences of membrane damage (Villiers, 1980; Coolbear, 1994). Viable seeds have the ability to repair limited ultrastructural damage during early imbibition but severe damage to the nuclei, such as condensation of chromatin material, is irreparable (Berjak and Villiers,1972). Parish and Leopold (1978) have reported that the rate of deterioration in soybean depends on the ability of the cell membranes to reform and function following the success of reorganisation and reparation mechanisms during rehydration.

Genetic damage

Genetic changes may induce mutations such as chromosomal aberrations in aged seed. Dourado and Roberts (1984) found that an increase in chromosome aberrations occur even with a small decrease in viability of barley and pea seeds. These can be lethal to the cell through impairment of DNA template function (Priestly, 1986). As seed deteriorates, DNA of the embryonic axis is damaged. Because of lack of DNA repair enzymes such as DNA ligases (Roberts and Black, 1989), this leads to deficiencies in mRNA production and subsequent translation of protein and enzymes (Ghosh and Chauduhri, 1984). Losses in capability to repair DNA damage can cause gene mutation which is heritable, or genes will be deleted from the meristem (Roos, 1980; Roberts, 1983). The effect of chromosomal aberrations on germination is reflected in the production of abnormal seedlings at the beginning of mitosis in root tips of germinating seeds (Roos, 1980).

2.2 Seed Testing methods for assessing seed quality

2.2.1 Purity analysis

The object of the purity analysis is to determine the composition by weight of the

sample and to identify the various species of seeds and inert particles constituting the sample of the seed lot. The seed lot analysed is separated into 3 components; pure seed, other seeds and inert matter, according to the International Seed Testing Association Rules (1993). "Pure seeds" include intact seeds of all varieties or species being analysed or immature, undersized, shrivelled, diseased, sprouted, and damaged seeds which are larger than half their original size. "Other seeds" include other crop or weed seeds found in the sample. "Inert matter" consists of material of no value, such as chaffy matter, leaves, bark, straw, earth particles, stones, nematode galls, and seed that has turned into fungal sclerotia, split seeds or separated cotyledons of legumes seed, broken or damaged seed of half the size or less. Seeds of legumes eg. peanut and mungbean, with the seedcoat entirely removed are regarded as inert matter (ISTA, 1993). High proportions of contaminants will lower seedlot quality. The presence of physical characteristics of seeds, such as seedcoat colour and broken cotyledons, the type of inert matter, and the uniformity of the seedlot can be used to assess the causes of poor seed quality. Inert matter may affect seed quality during storage because it presents sites for insect and fungal growth (Harrington, 1972).

2.2.2 Thousand seed weight

Thousand seed weight can be obtained by measuring the 100 seed weight of 8 individual replicates taken from the seedlot. Some references state that seed weight and/or seed size is correlated with seed germination, vigour and subsequent plant performance (Powell, 1988; Delouche, 1992), larger or heavier seeds being better in these record than smaller or lighter seeds within a seed population of some crop species, such as peanuts (Baskin and Delouche, 1971a) and soybean (Delouche, 1980).

2.2.3 Seed moisture content test

The moisture content of seeds can be measured by several methods, such as electric moisture meters, distillation methods, oven methods, etc., but only the use of the Air- Oven

method is recommended by the International Seed Testing Association Rules (1993). Although oven methods have the disadvantage of requiring considerable equipment, weighing of material, and considerable time for testing, they are accurate methods (Justice and Bass, 1978)

The oven methods operates on the principle that seed moisture is driven off by heat. However, it is important that only the free water, which is capable of moving freely from the inside of seed to seed surface, is removed and nothing else (no volatile materials, no oxidation and no decomposition). Seed samples are often ground to decrease drying time. The temperature and time of drying used depend on the kind of seed. Seeds which do not lose volatile constituents other than water at high temperature eg. maizes are dried at a temperature of 130°C for four hours, cereals are dried at a temperature of 130°C for two hours, or at 130°C for one hours in other crop seeds. However, seeds which lose volatile constituents at 130°C should be dried at 103°C for 17 hours, eg. peanut, soybean. The loss in weight of the seeds following heating is regarded as the moisture content (Justice and Bass, 1978).

2.2.4 Germination test

The aim of the germination test is to determine the maximum capacity of the seedlot to produce normal seedlings under optimum test conditions (moisture, aeration, temperature and light in some species), which can then be used to compare the quality of different seedlots. The test also provides information about the potential planting value of the seedlot. The germination test has been developed as a standardised test by the International Seed Testing Association (ISTA), and is internationally used to assess seed viability. After germinating, seedlings are counted and reported as the percentage of normal seedlings, abnormal seedlings, hard seeds which have not imbibed water during the test period, fresh ungerminated seeds and dead seeds. All these categories are based on the presence of essential structures including the root system, hypocotyl, epicotyl, cotyledons, and shoot

apex. Normal seedlings are those which show potential for continued development into normal plants when germinated, and which possess complete essential structures, i.e. a well developed root system and intact plumule, or show only slight defects, or are subjected to secondary fungal infection. Seedlings which do not have the capacity to develop into normal plants when germinated under favourable conditions, or show irreparable defects in essential parts are classified as abnormal seedlings (ISTA, 1979; ISTA, 1993).

Types of abnormal seedlings and causes

From the time of seed development in the field, through harvesting, threshing, processing and storage, seeds may be damaged by many factors. Such damage can cause loss in seed viability and germination, induction of abnormal seedlings and seed death. The different abnormal characteristics of seedlings that occur in the germination test can be classified into three major groups, i.e. damaged seedlings, deformed or unbalanced seedlings and decayed seedlings, based on the standards prepared by the International Seed Testing Association (ISTA, 1993). Thus, Islam (1984) and Koolkaew (1991) have used the types of abnormal seedlings present in standard germination test as a means of detecting previous history of seedlots or causes of poor quality.

Damaged seedlings

Damaged seedlings are seedlings with any of the essential structures missing or so badly damaged that balanced development cannot occur. These result from external causes, such as mechanical handling, heat, drought or insect damage. Such damaged seedlings include those with one or more of the following defective features (Wellington, 1970; ISTA, 1979; 1993).

i. Defective primary root, such as broken root or root split from the tip, short and stunted or blunt with insufficient secondary roots or no root at all, restricted root without root hairs.

ii. Stem or hypocotyl and epicotyl defects, due to deep cracking or breakage and splitting that damages the conducting tissues. Also the stem may be short or show no elongation or constriction.

iii. Cotyledons and primary leaf defects, such as breakage, splitting, missing or completely separated from the shoot axis by more than half of the original total tissues or no primary leaf development.

iv. Terminal bud breakage or no terminal bud

v. The whole seedling is weak or out of proportion compared with normal seedling, and shows poor development.

Deformed or unbalanced seedlings

Seedlings are abnormal when development as a whole is weak or unbalanced compared with that of a normal seedling germinated at the same time. Deformed seedlings may appear with one or more of the following characteristics (ISTA,1979; 1993).

i. Primary root that is stunted or stubby, retarded, constricted, curled, spindly, showing negative geotropism, or with weak secondary roots.

ii. Hypocotyls or epicotyls short and thick , constricted, bent over or looping, tightly twisted or spiralled.

iii. Cotyledons and primary leaf defects, such as curled or deformed, discoloured or necrotic with less than half of total leaf area left functioning, or emerging before the root. If primary leaves of *Phaseolus* and *Arachis* species are normal in shape but their size is smaller than one-fourth of original normal size they are also classified as deformed abnormal seedlings.

iv. Stunted or undeveloped terminal bud

v. Seedlings as a whole are weak (yellow or white seedlings), two seedlings fused together, or show inverted direction of growth.

Such deformed seedlings may be caused by internal disturbances of a physiological-

biological nature, which is often due to earlier external factors, such as unfavourable growing conditions of the parent plant eg. nutrient deficiency, poor weather conditions during seed ripening and harvesting such as high temperature and humidity, premature harvesting, poor threshing and processing procedures, chemical application effects, or inappropriate storage conditions. In some instances they may be the result of the genetic constitution or natural ageing of the seed (ISTA, 1979).

Decayed seedlings

Seedlings are classified as decayed when the essential structures and attachment areas are so diseased or decayed or rotten preventing normal development due to primary infection by fungi or bacteria (ISTA, 1979; 1993). If infection occurs on the cotyledons and/or primary leaves, the 50% rule is applied to classify the seedling i.e. if less than 50% of cotyledons and/or primary leaves are infected then the seedling is normal. However the tissues around the attachment of cotyledons to hypocotyl are important. If this area is affected then the 50% rule cannot apply and seedlings are classified as abnormal.

2.2.5 Accelerated ageing test

The performance differences between high germinating seedlots is ascribed to the result of seed vigour, which is a component of seed quality (Hampton and Hill, 1990; Hampton and Coolbear, 1990). Vigour of seedlots can be measured by several laboratory tests eg. accelerated ageing test, conductivity test, etc. An accelerated ageing test has been developed and accepted by ISTA to distinguish between seedlots of differing vigour levels, to estimate the storability of seed in storage, and to predict field planting potential (Delouche and Baskin, 1973; ISTA, 1987).

The principle of the accelerated ageing test is to expose seeds to a stress environment of high temperature and high relative humidity for a short period of time, and

then carry out the normal germination test. The stress condition imposed will cause rapid seed deterioration. High vigour seedlots will resist these extreme stress conditions better and deteriorate at a slower rate, and show higher percentage of normal seedling than low vigour seedlots.

The test provides a reproducible result which is more closely correlated with field performance for a range of crop species such as maize (Bruggink, 1989), mungbean, french bean, and soybean (Eua-umpon, 1991 and Hampton *et al.*, 1992) , including small seeded legumes (Wang and Hampton, 1989).

2.2.6 Conductivity test

Changes in the organisation of cell membranes occur during seed development prior to physiological maturity, during seed desiccation before harvest, and during imbibition prior to germination (Abul-Baki, 1980; Hampton, 1994b). As seeds begin to take up water during early imbibition, repair and reorganisation mechanisms of cell membranes occur, and allow solutes to leak from the seed tissues. In damaged or deteriorated or non-viable seeds, this solute loss may be much more severe due to an increase in membrane permeability, and hence the repair and reorganisation process of membrane cells cannot cope with too rapid an influx of water and an increase in enzymatic activity of the cells (Matthews and Powell, 1981; Crowe *et al.*, 1989; Coolbear, 1994). The leakage of solutes during imbibition is a good indication of the health of seed tissues or degree of membrane deterioration and thus, by inference, seed vigour.

The electrolytes leaking from seed tissues can be measured by the electrical conductivity test. The test has been developed as a routine vigour test to predict field emergence in garden pea and other legumes (Hampton, 1994b; Hampton and Tekrony, 1995). The leakage is a reflection of the level of seed coat cracking or membrane permeability, the rate of membrane reorganisation within the seed, and the amount of

organic (sugars and amino acids) and inorganic ions (eg. potassium) in the cells (Simon and Raja Harun, 1972; Coolbear, 1994). Thus, the conductivity test can be used to predict seed performance when membrane damage is the most likely cause of loss of seed vigour. Low vigour seeds normally possess poor membrane structure, such as in damaged or deteriorating seed, and will lose considerable amounts of solutes resulting in high conductivity measurements. In contrast, high vigour seeds show minor electrolyte leakage (Simon and Raja Harun, 1972; Powell and Matthews, 1981; Hampton, 1994b).

2.2.7 Health tests

As seed deteriorates or loses germinability and viability, field and storage fungi may become involved. Field fungi or seedborne fungi can cause pod and seed diseases, seed rot, pre-and post emergence damping-off, seedling stunting or abnormalities, and reduce germination of seed. Storage fungi play a major role in seed deterioration in storage, particularly under poor conditions. Seed health testing, is used to detect the presence of seed-borne fungi in seedlots and the results can be used to compare the value of different seedlots (Agarwal and Sinclair, 1987). Blotter and agar plate tests are recommended by ISTA for the routine examination of crop seeds for fungal infection.

The blotter test, where seeds are germinated on damp blotters, is often difficult to evaluate and to identify the fungal types infecting seeds in legumes, eg. beans, because the germination character of the seed lifts the seed coat and cotyledons, from the blotter surface (Agarwal and Sinclair, 1987). In the agar plate test, seeds are surface sterilised to remove contaminants and then planted on a sterile agar medium. Identification is based on the growth and colony characteristics occurring on the nutrient medium, usually malt extract agar or potato-dextrose agar, for detection of the seedborne fungi. For identification of storage fungi it is usual to use an agar medium containing salt (sodium chloride) because this medium tends to restrict fungal growth so that fungi from adjacent seeds do not over grow one another (Christensen, 1973; Agarwal and Sinclair, 1987).

CHAPTER 3
MATERIALS AND METHODS

Five seedlots of different cultivars of mungbean (lot 1 cv. chinese, lot 2 cv. Berken, lot 3 cv. Regur, lot 4 cv. Berken and lot 5 unknown cultivar) from Seedbank (NZ) Ltd. and three seedlots of different cultivars of peanut (lot 1 cv. Spanish Red, lot 2 cv. Virginia and lot 3 cv. Spanish White) from Australia in 1992 were used in this study.

3.1 EXPERIMENT A QUALITY EVALUATION

Subsamples of 150 g of mungbean and 1 kg of peanut were randomly drawn from the whole seedlot using soil divider (ISTA, 1993). All seedlots were tested for initial seed quality using the following laboratory methods.

3.1.1 Purity analysis

Purity working samples of 120 g for mungbean and 1 kg for peanut were randomly drawn from each seedlot using a soil divider. The working sample of each seedlot was then individually sorted into pure seed, other seeds, and inert matter according to International Seed Testing Association (ISTA) Rules (1993). These three components were weighed and expressed as a percentage of the total weight, and the pure seed was used for the further tests.

3.1.2 Thousand seed weight

From the pure seed fraction, seeds were randomly counted by hand into four replications of 100 seeds and each weighed. The seed weight was then calculated to 1000 seed weight and recorded in grams.

3.1.3 Seed moisture content test

Determinations of seed moisture content of seedlots were conducted by using the hot air oven method as prescribed in the ISTA Rules (1993). Four replications each of 10 g were sampled from the pure seed. Seed samples were ground (for mungbean) or quick cut (for peanut) and put in preweighed aluminium containers, then weighed and dried in an air oven at 130°C for 1 hr for mungbean, and at 103°C for 17 hr for peanut seed. After drying, the samples were cooled in desiccators for about 30-45 minutes and then reweighed (two decimal places). The percentage of moisture content was calculated on a fresh weight basis by using the following formula:

$$MC = M_2 - M_3 \; x \; \frac{100}{M_2 - M_1}$$

where

M_1 is the weight in grams of the container and its cover,

M_2 is the weight in grams of the container and its cover and its contents before drying, and

M_3 is the weight in grams of the container and its cover and its contents after drying.

3.1.4 Standard germination test

Four replications each of 50 pure seeds were taken at random from the seedlots. The between paper method (B.P.) was used for mungbean seeds and the sand method was used for peanut seeds (ISTA,1993). Peanut seeds were pretreated with a fungicide (thiram), about 5 mg per replication, to avoid fungal growth before evaluation of the germination test.

Between paper (B.P.) method for mungbean

Mungbean seeds were placed between two sheets of damp paper and the paper then was rolled up and secured with a rubber band. The rolled samples were then put into wire baskets, enclosed in large plastic bags to retain moisture for germination. Germination tests were carried out at a temperature of 20°C for up to 7 days. First and final counts were taken on the 4th day and the 7th day, respectively. At the first count, normal seedlings, abnormal mouldy seedlings and dead mouldy seeds were removed and recorded. All other seeds and seedlings were left until the final count at which stage all categories of seedlings, ungerminated seeds and dead seeds was recorded.

The categorisation into normal seedlings, abnormal seedlings, and dead seeds was based on the ISTA Rules (1993). Germination was expressed as the percentage of normal seedlings, abnormal seedlings, and dead seed.

Sand (S) test method for peanut

Moist sand (2.5 cm deep) was put into plastic containers. Fungicide treated peanut seeds were then placed on sand surface and covered with a further 1-2 cm of moist sand. The containers were kept in a cabinet of high humidity and temperature at 25°C for up to 10 days. Peanut seedlings were then evaluated following the ISTA rules on the 10th day. Germination was recorded as the percentage of normal seedlings, abnormal seedling and dead seeds.

3.1.5 Accelerated ageing test

The accelerated ageing test (AA) was carried out according to the method prescribed in the ISTA vigour testing handbook (1987). The basis of this test was to expose seeds to extreme stress conditions of high relative humidity and high temperature for a short period of time.

Four replications of 30 g of mungbean or 60 g of peanut from each seedlot were separately placed on a wire mesh tray, placed in a plastic box (11.0 x 11.0 x 3.5 cm) containing 40 ml of distilled water to provide a humidity of approximately 100%, and covered with a secure lid. The containers with samples then put in the ageing chamber spaced approximately 2.5 cm apart and incubated at 42°C for 72 hr for mungbean (ISTA,1987) and at 45°C for 24 hr for peanut (pre-test). After ageing, 50 aged seeds were tested for germination), and 10 g of seeds tested for moisture content using the oven method to examine the accuracy of the test.

3.1.6 Conductivity test

The conductivity test was carried out according to the method prescribed in the ISTA vigour testing handbook (1987). Four replications of 50 seeds within range of 10-14% seed moisture content were used from each seedlot of mungbean and peanut. Each replication was weighed (two decimal places) before being put into a 500 ml flask containing 250 ml of distilled water which was equilibrated at 20°C for mungbean seed and 25°C for peanut seed for 24 hours. The top of each flask was then sealed with parafilm to prevent evaporation and contamination. A control flask containing only distilled water was also prepared. All samples and control flasks were kept for 24 hours at 20°C for mungbean and at 25°C for peanut. At the end of the soak period, the conductivity of the solution was measured immediately . The control flask and each flask with soak seeds were swirled for 10-15 seconds and conductivity determined by immersing the dip-type cell of the electrical conductivity meter into the solution. The conductivity reading of each sample was subtracted by the reading from the control (distilled water) reading and then calculated as conductivity (μs) per gram of seed weight as follows:

$$\frac{conductivity(\mu s)\ for\ each\ flask}{weight\,(g)\ of\ dry\ seed\ sample} = \mu s\ cm^{-1}g^{-1}$$

3.1.7 Seed health test

The agar plating method was used to determine fungal infection. The media used were potato-dextrose agar (PDA) for field fungal detection and potato-dextrose agar plus salt (PDAS) for storage fungal detection. PDA was prepared by dissolving 39g of potato-dextrose agar in 1 litre of distilled water and 0.05g of Chloramphenical and for PDAS media 75 grams of salt was added. All media were sterilised in an autoclave at 121 °C and 15 psi for 20 minutes. The seeds were surface sterilised for 5 minutes by soaking in a 1-2% sodium hypochlorite solution (1 part of 'Janola' : 3 parts of water) then rinsed in running water for 3 minutes. Four replications of 10 seeds were placed on the agar surface in PDA plates and PDAS plates (10 seeds per plate for mungbean and 5 seeds per plate for peanut). and then incubated at 25 °C for 7 days. The number of infected seeds were recorded when fungal colonies and spore characteristics were sufficiently developed for identification. Percentages of field and storage fungi were then calculated.

3.2 EXPERIMENT B SEED STORAGE

On the basis of the earlier experimental results, the seeds of the following three mungbean seedlots and one peanut seedlot with high germination percentage and vigour were selected for storage studies: mungbean lot 1 cv. Chinese, lot 2 cv. Berken and lot 3 cv. Regur, and peanut lot 3 cv. Spanish White. Other lots were not used due either to poor quality or insufficient seed.

3.2.1 Storage conditions

To study the storage performance of mungbean and peanut seedlots of different initial quality under different storage conditions, various storage conditions were created by:

a) adjusting seed moisture content to a suitable level for sealed storage (8.6% for

mungbean and 6.6% for peanut) or at a higher level (13.5% for mungbean and 11.5% for peanut),

b) storing mungbean seeds in open and sealed containers for up to 8 months in two different temperature/relative humidity combinations (20°C/75%RH and 30°C/95%RH), and

c) storing peanut seeds in open and sealed containers for up to 8 months in three different temperature/relative humidity combinations (5°C/85%RH, 20°C/75%RH and 30°C/50%RH), and in open containers at 30°C/95%RH.

3.2.2 Adjusting seed moisture contents

Each seedlot was divided into 4 sublots by using a soil divider. Each sublot was divided into two portions by using the same divider. The first portion was dried to a low seed moisture content (SMC) considered a suitable seed moisture content for sealed storage (8.6 %SMC for mungbean, and 6.6 % for peanut). The second portion was adjusted to a higher seed moisture content (13.5% SMC in mungbean and 11.5% SMC in peanut).

To raise seed moisture content, seed samples of known moisture and weight were placed in a single layer on wire mesh tray above water in an air tight container (Loeffler *et al.*, 1988) at 5°C for equilibration and by frequent weighing until the desired moisture content was achieved as using the formula:

$$W_2 = \frac{100-A}{100-B} \; x \; W_1$$

where A = initial seed moisture content(%)

B = desired seed moisture content(%)

W_1 = initial weight of seeds (g)

and W_2 = the required weight of seeds (g).

To reduce seed moisture content, seed samples in cloth bags were placed over dry silica gel in desiccators for varying times until the desired weight (seed moisture content) was obtained as calculated by the above formula.

After attaining the desired moisture contents, about 18 g of mungbean seeds and 100 g of peanut seeds at each SMC level were separately placed in different types of containers, *ie.*

a) muslin cloth bags tied at the open end, and

b) laminated aluminium foil packets heat sealed.

The laminated aluminium foil packets which were moisture proof containers were used for `sealed' storage, while cloth bags which moisture migration can occur were used as `open' storage.

Forty packets of each lot of mungbean seeds at 8.6%SMC and 40 packets at 13.5%SMC were prepared to allow five evaluation times during an eight months storage under two environmental conditions, except that for open storage only 36 bags were prepared because there was not enough seed. The samples of three mungbean seedlots for two storage conditions totalled 240 sealed packets and 216 open bags. The single lot of peanut was prepared into 60 sealed packets and 80 open bags for each of the two seed moisture content levels resulting in 120 sealed packets and 160 open bags.

Each storage condition of temperature/relative humidity was treated as separate experiment using separate cabinets. In each storage environment a randomized complete block design was used by randomly placing seed samples within a block of four replications.

The treatments for each mungbean seedlot and peanut were therefore as follows:

Mungbean

[20°C/75%RH] [8.6% SMC] [sealed storage]
x x x 4 replications x 5 sampling time
[30°C/95%RH] [13.5% SMC] [open storage]

Peanut

[5°C/85%RH] [6.6% SMC] [sealed storage]
[20°C/75%RH] x x x 4 replications x 5 sampling time
[30°C/50%RH] [11.5% SMC] [open storage]
and (only open storage) at 30°C/95%RH.

Samples in each treatment were withdrawn randomly from the storage cabinet at intervals of 1, 2, 4, 6, and 8 months to evaluate seed quality using the following tests, except for the 30°C/95%RH treatment where insufficient seed prevented a sampling at 8 months.

1. Seed moisture content test,
2. Standard germination test,
3. Conductivity test,
4. Fungi test,

using the same methods described in experiment A.

After the first month storage of mungbean seed samples, the oven malfunction occurred in the at 30°C 95%RH treatment. As a result stored mungbean results in this treatment at one month should be treated with caution. Peanut seeds stored at 30°C 95%RH were seriously deteriorated and overgrown by storage fungi after 2 months, and samples were therefore not tested for quality.

CHAPTER 4
RESULTS

4.1 EXPERIMENT A SEED QUALITY EVALUATION

4.1.1 MUNGBEAN

The results from different laboratory tests used to determine the initial quality of five mungbean Seidlitz is presented in Table 1.

4.1.1.1 Purity analysis

The purity percentages of 5 seedlots of mungbean ranged from 97.4 to 99.8 %. Seedlot 4 had the lowest purity, and lot 3 had the highest purity. However, the pure seed in lot 1 contained a small percentage of brown and shrivelled seed, and seeds with a wrinkled seed coat. Approximately 4.6% of physically damaged seeds, varying from surface damaged to severely impacted seed was also found in seedlot 1 when 400 pure seeds were examined for visible damage. There was only a small percentage of visible damaged seeds in lots 2 and 4, and only a trace (less than 0.05%) in other seedlots, however, pure seeds in lots 2 and 4 contained a small percentage of slightly wrinkled and unclean seed coats. The inert matter fraction (particularly in lot 4) mainly comprised of a small percentage of broken and split seeds which were less than half of the original seed size.

4.1.1.2 Thousand seed weight

The five seedlots of mungbean showed differences in 1000 seed weight, ranging from 57.5 g to 63.8 g. Seedlots 3 and 5, which were larger, had higher 1000 seed weight than other seedlots.

Table 1 Initial quality of five mungbean seedlots. Data presented are means ± S.E

Lots	Purity (%)		TSW	SMC	Germination (%) before AA. test			Germination (%) after AA. test			COND	FF	SF
	Pure seed	Inert matter	(g)	(%)	NORM	ABN	DEAD	NORM	ABN	DEAD	(μs/cm/g seed)	(%)	(%)
1	99.1	0.7	58.3 ±1.1	12.9 ±0.1	88 ±4	5 ±1 [1]	7 ±3	55 ±3	35 ±7	10 ±5	31.4 ±2	3	3
2	99.5	0.5	58.3 ±0.5	11.5 ±0.1	94 ±3	6 ±3 [2]	0.00	51 ±3	39 ±5	10 ±2	26.0 ±1	25	5
3	99.8	0.2	63.8 ±0.3	12.2 ±0.1	94 ±2	6 ±2 [3]	0.00	66 ±6	21 ±4	13 ±3	22.9 ±1	13	13
4	97.4	2.6	57.5 ±0.7	10.9 ±0.1	86 ±2	12 ±2 [4]	2 ±3	35 ±13	47±10	18 ±2	26.5 ±1	15	5
5	99.0	1.0	62.0 ±1.1	18.7 ±0.1	80 ±4	15 ±5 [5]	5 ±4	18 ±6	35 ±8	47 ±11	40.8 ±4	15	88

Note TSW = Thousand seed weight (g) SMC = Seed moisture content (%) AA. =Accelerated ageing test NORM = Normal seedling (%)

ABN = Abnormal seedling (%) DEAD = dead seed (%) COND = Conductivity (μs/cm/g seed)

FF = Field fungi (data presented are % of infected seeds from 40 seeds) SF = Storage fungi (data presented are % of infected seeds from 40 seeds)

1 = Seedling abnormalities included **2% damaged** seedlings (broken cotyledons and broken primary root with insufficient secondary root) and **3% decayed** seedlings.

2 = Seedling abnormalities included **2% damaged** seedlings (deep cracked hypocotyl, broken root or cotyledon and primary leaves) and **4% deformed** seedlings (radical emerged seedling, stunted root, and stunted root, short thin and curled hypocotyl.

3 = seedling abnormalities included **1% damaged** seedlings (broken cotyledons and plumule) and **5% deformed** seedlings (stunted root, emerged radicle seedling).

4 = seedling abnormalities included **5% damaged** seedlings (broken root and/or cotyledons and plumule, broken root with insufficient secondary root, and deep cracked hypocotyl), **4% deformed** seedlings (stunted root, radicle emerged seedling, curled hypocotyl with no root) and **3% decayed** seedlings.

5 = seedling abnormalities included **9% deformed** seedlings (stunted root, stunted root and hypocotyl and/or curled hypocotyl, and weak seedlings) and **6% decayed** seedlings.

4.1.1.3 Seed moisture content

The five mungbean seedlots contained different levels of seed moisture, ranging from 10.9% to 18.7%. Lot 5 had the highest moisture content while lot 4 had the lowest level.

4.1.1.4 Standard germination test

There was a difference between seedlots in terms of initial percentage of normal seedlings ranging from 80% to 94% after seed had been germinated at 20°C for 7 days, being highest in lots 2 and 3 and lowest in lot 5. This latter also produced the highest percentage of abnormal seedlings and a relatively high level of dead seed. The two lowest germinating seedlots (4 and 5) also produced the highest level of abnormal seedlings.

Types of seedling abnormalities

The major types of abnormal seedlings evaluated in the germination test were categorised into damaged, deformed and decayed abnormal seedlings as prescribed in the ISTA Rules (1993). The level of each abnormality varied between lots (Table 1). Lot 4 had higher level of damaged seedlings (5%) than other lots (1-2%), while Lot 5 had the highest level of deformed (9%) and decayed seedlings (6%). It was interesting that lot 5 had no 'damaged' abnormals, lot 2 and lot 3 had no 'decayed' abnormals and lot 1 had no 'deformed' abnormals, only lot 4 produced abnormal seedlings of all three types.

4.1.1.5 Accelerated ageing test

Accelerated ageing (AA) allowed comparisons to be made between lots in terms of vigour ranking. The seedlots were exposed under accelerated ageing conditions of high relative humidity (about 100%) and high temperature (42°C) for a period of 72 hrs.

All seedlots showed a reduction in germination after accelerated ageing. Lots 4 and 5 in particular were seriously deteriorated by accelerated ageing with germination dropping by more than 60% in lot 5. The comparison in AA reaction between lots 1 and 4 is particularly interesting. Both had similar germination prior to AA test (86 and 88%). However, after AA exposure germination fell to 35% in lot 4 compared with 55% in lot 1, as a result of increased abnormal seedlings and dead seed.

4.1.1.6 Conductivity

The measurement on solute leakage of different mungbean seedlots after soaking in distilled water for up to 24 hours showed that the 5 seedlots had different conductivity readings ranging from 22.9 to 40.8 μs/cm/g seed. Seedlot 5 showed the highest conductivity reading while seedlot 3 showed the lowest reading. This result is entirely consistent with the respective initial germination and accelerated ageing performance of these two seedlots.

4.1.1.7 Seed health test

There was variation in the levels of infection of different seedlots with field and storage fungi. Results are presented in Tables 1 and 2.

Table 2 Occurrence of different field and storage fungal genera in five mungbean seedlots. Data presented are percentage of fungal colonies from 40 seeds.

Lot	Field fungi (%)			Storage fungi (%)			
	Alternaria spp.	Other genera	Total	*Aspergillus spp.*			
				A. glaucus	*A. flavus*	*A. candidus*	Total
1	3	0	3	0	3	0	3
2	18	8	26	0	5	0	5
3	13	0	13	13	0	0	13
4	13	3	16	0	5	0	5
5	10	5	15	68	28	3	99

Note Other genera were predominantly *Fusarium spp.* and *Cladosporium spp.*

Field fungi

Percentage infection by field fungi in 5 mungbean seedlots were tested on potato dextrose agar (PDA) incubated at 25°C for 7 days, and showed highest levels of infected seeds in lot 2, and lowest in lot 1. The most common genera of field fungi found on the media of all seedlots was *Alternaria spp.* Other genera, such as *Fusarium spp.*, and *Cladosporium spp.* were present at low levels or absent.

Storage fungi

The infection of storage fungi on 5 mungbean seedlots detected on potato dextrose agar plus salt (sodium chloride), showed that initially 5 seedlots of mungbean have been infected by storage fungi at different levels. Lot 5 was severely infected by storage fungi (approximately 88%), while only 13% of seeds in lot 3 and a small percentage in the other lots were infected. The only genus of storage fungi found was *Aspergillus*, predominated in lots 3 and 5 by *Aspergillus glaucus* , and in lot 5 also *by Aspergillus flavus.*

4.1.2 PEANUT SEED

The results from different laboratory tests used to determine the initial quality of three peanut seedlots is presented in Table 3 : lot 1 cv. Spanish red, lot 2 cv. Virginia and lot 3 cv. Spanish white.

4.1.2.1 Purity analysis

The purity percentages of 3 seedlots of peanut ranged from 91.2 to 95.7 %. Seedlot 3 had the lowest pure seed percentage, while lot 2 had the highest pure seed. However, the pure seed of lot 1, and in particularly lot 3 contained a small percentage of brittle and fragile coated seeds. The inert matter in all lots, but particular lot 3, was mostly dust, broken testa without seed, seeds and pieces of seed without testa, and

Table 3 Initial quality of three peanut seedlots. Data presented are means ± S.E.

Lots	Purity (%)		TSW	SMC	Germination (%) before AA. test			Germination (%) after AA. test			COND	FF	SF
	Pure seed	Inert matter	(g)	(%)	NORM	ABN	DEAD	NORM	ABN	DEAD	(μs/cm/g seed)	(%)	(%)
1	94.3	5.7	522 ±8	7.4 ±0.1	57 ±7	43 ±7 [1]	0.00	63 ±1	30 ±6	8 ±7	19.6 ±3	20	83
2	95.7	4.3	739 ±11	8.2 ±0.1	43 ±8	38 ±11 [2]	20 ±10	28 ±3	35 ±4	37 ±3	24.6 ±1	20	98
3	91.2	8.8	472 ±11	6.6 ±0.1	72 ±2	27 ±1 [3]	1 ±2	67 ±6	27 ±7	6 ±2	12.9 ±1	18	43

Note TSW = Thousand seed weight (g) SMC = Seed moisture content (%) AA. = Accelerated ageing test NORM = Normal seedling (%)
ABN = Abnormal seedling (%) DEAD = dead seed (%) COND = Conductivity (μs/cm/g seed)
FF = Field fungi (data presented are % of infected seeds from 40 seeds) SF = Storage fungi (data presented are % of infected seeds from 40 seeds)

1 = Seedling abnormalities included **1% damaged** seedlings (deep cracked hypocotyl), **27% deformed** seedling (weak plumule, no terminal bud, insufficient root and thick stunted hypocotyl and seedling with radicle emerged but no growth) and 15% decayed seedlings (root and hypocotyl rot and/or severe fungal infected on cotyledon and shoot).

2 = Seedling abnormalities included **2% damaged** seedlings (deep cracked hypocotyl or root), **28% deformed** seedling (weak plumule, insufficient root and thick stunted and/or curled hypocotyl and seedling with radicle emerged but no growth) and **8% decayed** seedlings (root and hypocotyl rot and/or severe fungal infected on cotyledon and shoot).

3 = Seedling abnormalities included **2% damaged** seedlings (cracked root and hypocotyl) **20% deformed** seedlings (weak plumule, insufficient root and thick stunted and/or curled hypocotyl and seedling with radicle emerged but no growth) and **2% decayed** seedling.

broken and split seeds which were less than half of the original seed size. No weed seed or other crop seed contaminants were found in any of the 3 peanut seedlots (Table 3).

4.1.2.2 Thousand seed weight

The three seedlots of peanut showed a difference in 1000 seed weight, ranging from 472 g to 739 g. Seedlot 2 (cv. Virginia) which was largest in seed size had highest 1000 seed weight, while seedlot 3 (cv. Spanish white) which was smallest had lowest 1000 seed weight (Table 3).

4.1.2.3 Seed moisture content

The three seedlots of peanut were at a different levels of seed moisture content, ranging from 6.6% in lot 3 to 8.2% in lot 2 (Table 3).

4.1.2.4 Standard germination test

There was a difference between seedlots in terms of initial percentage of normal seedlings ranging from 43% to 72% after germination in sand at 25°C for 10 days (Table 3). Seedlot 3 had highest percentage of normal seedlings and lowest abnormal seedling and dead seed percentages. Seedlot 2 had the lowest normal seedling percentage, but highest levels of abnormal seedlings and dead seeds. Although seedlot 1 had lower germination than lot 3 , no dead seed were found at the conclusion of the germination test.

Types of seedling abnormalities

The major types of abnormal seedlings found in germination tests of the three peanut seedlots were 'deformed' and 'decayed' seedlings. The level of each type varied between lots (Table 3). Lots 1 and 2 had similar levels of deformed seedlings (27-28%) but lot 2 had a lower level of decayed seedlings (8%) than lot 1 (15%). However, both

lots 1 and 2 had higher levels of deformed and decayed seedlings than lot 3 (20%). Levels of 'damaged' seedlings were low (1-2%) in all lots. Nevertheless all three types of abnormal seedlings occurred in all lots.

4.1.2.5 Accelerated ageing test

When the three seedlots of peanut were exposed to the extreme stress of high relative humidity (about 100%) and temperature (45°C) for a period of 24 hrs, germination capacity of seed, particularly in lot 2 were greatly decreased. Compared with standard germination before accelerated ageing, the results showed that the ranking order in germinability of the 3 seedlots after accelerated ageing was similar to that obtained following standard germination testing prior to accelerated ageing. The germination after aging showed an obvious increase in dead seed in lot 2, while seedlots 1 and 3 showed a small increase (Table 3).

4.1.2.6 Conductivity

The measurement on solute leakage of different peanut seedlots after soaking in distilled water for 24 hours showed that the 3 seedlots had different conductivity reading ranging from 12.9 μs/cm/g seed (lot 3) to 24.6 μs/cm/g seed (lot 2) (Table 3).

4.1.2.7 Seed health test

There was a variation in the levels of seed infection by field and storage fungi in three seedlots of peanut as presented in Tables 3 and 4.

Field fungi

Percentages of infection with field fungi in 3 peanut seedlots were obtained following seed placement onto potato dextrose agar (PDA) at 25°C for 7 days. No major differences in total infection occurred between seedlots but lots 1 and 2 were

more dominated by *Fusarium spp* than lot 3. The most common genera of field fungi found on the media of all seedlots were *Fusarium spp.* and *Rhizoctonia spp.* while there was a small percentage of other genera which was unidentified.

Storage fungi

The infection of storage fungi in 3 peanut seedlots was detected by placing seed onto potato dextrose agar plus salt (sodium chloride) and incubating at 25°C for 7 days. All seedlots were infected by storage fungi, with lots 1 and 2 being most severely infected (approximatly 88% and 124%, respectively), infection level was lower in lot 3 (48%). The most common genus of storage fungi found in lot 1 was Penicillium spp. whereas Aspergillus spp. predominated in the other two lots.

Table 4 Occurence of different field and storage fungal genera in three peanut seedlots. Data presented are percentage of fungal colonies from 40 seeds.

	Field fungi (%)				Storage fungi (%)				
Lot	*Fusarium*	*Rhizoctonia*	Other	Total	*Aspergillus spp.*			*Penicillium*	
	spp.	*spp.*	genera		*A. glaucus*	*A. flavus*	*A. niger*	*spp.*	Total
1	18	3	0	21	5	13	5	65	88
2	15	3	3	21	55	28	18	23	124
3	8	8	3	19	23	10	5	10	48

Note Other genera are unidentified fungi.

Differences in the species of *Aspergillus* between lots is of interest and may be important in suggesting the likely extent of deterioration in each lot. The 'pioneer' species *Aspergillus glaucus* was present in all lots but was particularly prevalent in lots 2 (55%) and 3 (23%). Species more commonly associated with the later stages of storage fungus infection in seed - *Aspergillus niger* and *Aspergillus flavus* - were associated with all lots, but were detected at higher levels in lots 1 and 2 (*A. flavus*) and lot 2 (*A. niger*).

4.2 EXPERIMENT B SEED STORAGE

4.2.1 The performance of mungbean seedlots under different storage conditions

Following initial seed quality tests, three seedlots of mungbean (lot 1 cv. Chinese, lot 2 cv. Berken and lot 3 cv.Regur) with high germination and vigour were selected for this experiment in order to study storage performance and deterioration rate of mungbean seed, after adjustment of seed moisture content to 8.6% or 13.5% and then stored under different storage conditions of different packaging (open and sealed storage), and temperature/relative humidity environments of 20°C/75%RH and 30°C/95%RH.

4.2.1.1 Seed moisture content (SMC)

The performance of the three mungbean seedlots in terms of seed moisture content when stored at 2 different initial seed moisture contents in open or sealed packaging for up to 8 months at 20°C/75%RH or 30°C/95%RH are presented in Table 5.

The three seedlots were stored at two different initial seed moisture contents (8.6% or 13.5%) in all treatments. However, during storage, the seed moisture contents changed in response to the effects of packaging, and storage temperature/relative humidity conditions.

Storage container highly influenced seed moisture content both at 30°C/95%RH and 20°C/75%RH. In sealed storage, seed stored at 8.6% and 13.5% initial seed moisture content in both storage environments showed slight but not appreciable changes in SMC from the initial level with increasing storage time, and there was no effect of temperature on seed moisture content between sealed storage at 20°C and 30°C. In open storage, however, SMC changed markedly from initial levels to reach equilibrium with the relative humidity of the storage environment. At 30°C/95%RH,

Table 5 Effects of different seedlots, initial moisture contents of 8.6% and 13.5% , packaging containers (open and sealed containers) on percentage of seed moisture content of mungbean after storage at intervals of 0, 2, 4, 6, and 8 months at 20°C/75%RH and 30°C/95% RH. Data presented are means of four replications.

Lot	Treatments																
	% ISMC	30°C/95%RH								20°C/75%RH							
		Time	Open			Sealed				Open				Sealed			
		0	2	4	6	2	4	6	8	2	4	6	8	2	4	6	8
1	8.6	8.6	17.0	23.8	26.3	9.0	9.1	9.3	9.2	12	11.9	12.2	12.1	9.0	9.2	9.3	9.2
	13.5	13.5	18.4	23.7	25.7	13.6	13.8	14.1	13.8	12.7	12.6	12.9	12.7	13.8	13.9	13.9	14.0
2	8.6	8.5	17.0	23.4	25.1	9.0	9.1	9.2	9.2	11.1	11.1	11.6	11.3	9.0	9.1	9.2	9.2
	13.5	13.3	17.7	23.7	24.6	13.4	13.5	13.5	13.7	12.0	11.9	12.2	11.9	13.3	13.6	13.8	13.4
3	8.6	8.6	15.8	24.8	25.8	9.0	9.2	9.3	9.3	12.0	11.9	12.3	12.1	9.0	9.2	9.4	9.3
	13.5	13.5	15.0	24.4	26.5	13.6	13.7	13.8	13.8	12.5	12.3	12.8	12.5	13.5	13.7	13.8	13.6

Note: ISMC = Initial seed moisture content (%).
RH = Relative humidity (%)
Time = Storage period (months)

both seed stored at 8.6% and 13.5% initial moisture content showed a marked increase in SMC in all three seedlots after 2 months storage, but at a slower rate in seedlot 3. This increase continued to approximately 24-26% after 6 months storage at 30°C/95%RH. Under 20°C/75%RH storage conditions, seed at 8.6% initial moisture content increased to approximately 12% SMC in seedlots 1 and 3, and 11.1% in seedlot 2 after 2 months storage and thereafter showed only slight fluctuation through to 8 months. Seed stored at 13.5% initial moisture content showed a noticeable decrease in SMC to about 12.7% in seedlot 1, 12.0% in seedlot 2 and 12.5% in seedlot 3 at 2 months and thereafter showed only minor fluctuation through to 8 months.

The difference between equilibrium moisture content levels in open storage depended on whether seed was absorbing moisture from dry initial levels or desorbing moisture into drier surrounding air - the well known ' hysteresis effect '. These results show there was no effect of initial seed moisture content on changes in seed moisture content in open storage at the higher temperature/relative humidity of 30°C/95%RH, but at the lower temperature/relative humidity of 20°C/75%RH seed stored at 13.5% initial seed moisture reached a higher (0.4-0.6%) level of equilibrium moisture content than seed stored at 8.6% initial moisture content.

4.2.1.2 Germination

Normal seedlings

As shown in Table 6 and Plate 1-3, the three seedlots of mungbean did not differ greatly in initial normal seedling percentage before storage, but germination was higher in seedlots 2 and 3 than in seedlot 1. However, these seedlots reacted differently to storage conditions and time when stored under different packaging systems, initial seed moisture contents, and temperature/ relative humidity combinations.

Storage at 30°C/95%RH had a particularly deleterious effect on seed germination in open storage, all seeds being dead after 6 months. However, seed stored

Table 6 Effects of different seedlots, initial moisture contents of 8.6% and 13.5% , packaging containers (open and sealed containers) on percentage of normal germination of mungbean after storage at intervals of 0, 2, 4, 6, and 8 months at 20°C/75%RH and 30°C/95% RH. Data presented are means of four replications.

Lot	% Initial NORM	Treatments															
			30°C/95%RH							20°C/75%RH							
		%	Open			Sealed				Open				Sealed			
		ISMC	2	4	6	2	4	6	8	2	4	6	8	2	4	6	8
1	88	8.6	55	2	0	81	82	87	81	84	85	84	83	84	83	84	85
		13.5	50	1	0	80	79	74	76	86	82	83	82	84	83	82	81
2	94	8.6	60	7	1	89	93	92	86	90	86	91	89	88	86	86	91
		13.5	53	3	0	91	83	87	80	89	91	91	88	88	88	90	88
3	94	8.6	91	3	0	98	95	96	96	94	97	95	97	97	93	94	95
		13.5	91	3	0	96	94	94	91	97	96	95	95	95	94	96	95

Note: NORM = Normal seedlings (%)
ISMC = Initial seed moisture content (%).
RH = Relative humidity (%)
Time = Storage period (months)

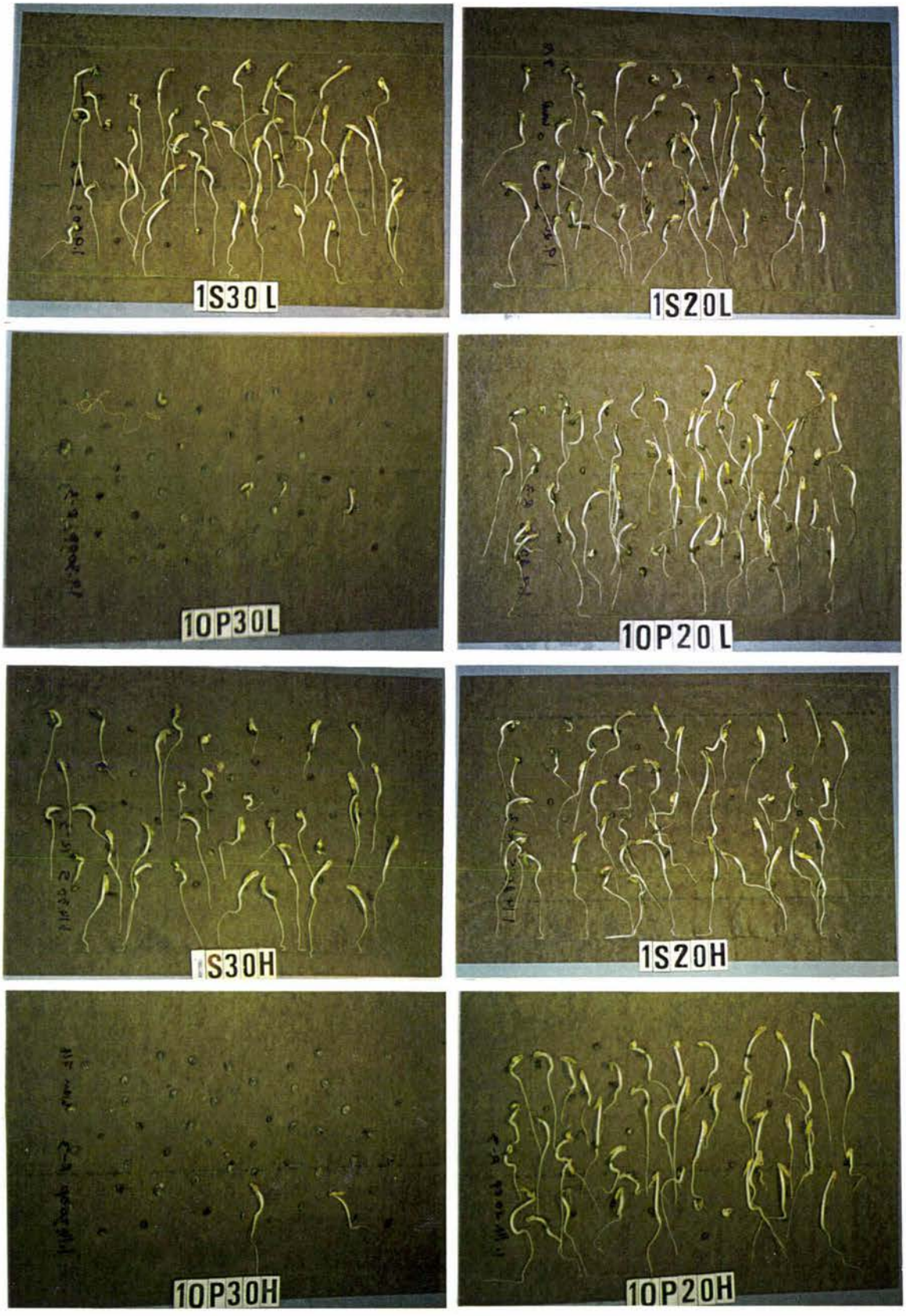

Plate 1 Five day old seedlings of mungbean from seed lot 1 after 6 months storage in open and sealed containers at 8.6% (L) and 13.5% (H) initial seed moisture contents at 30°C/95%RH and 20°C/75%RH.

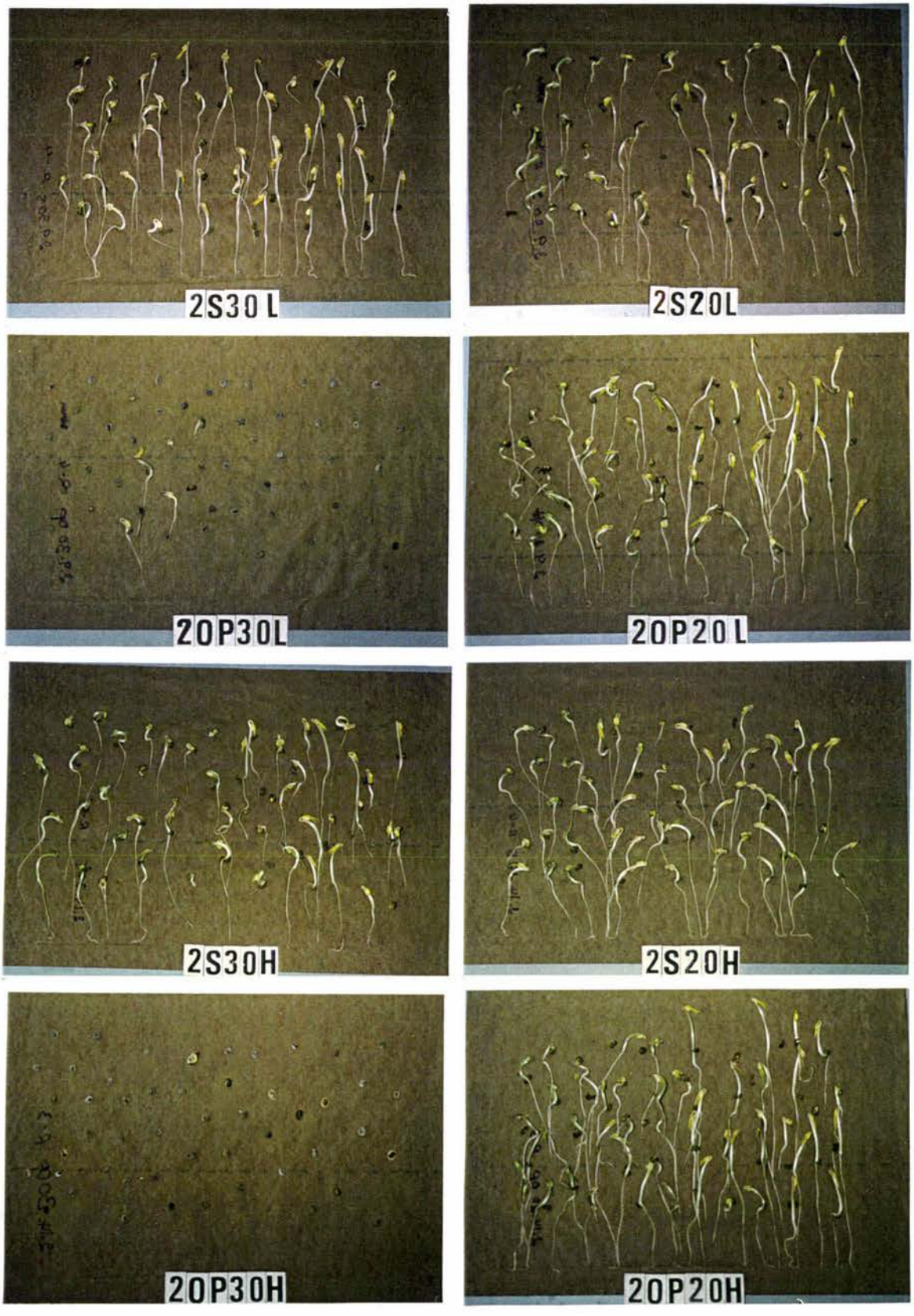

Plate 2 Five day old seedlings of mungbean from seed lot 2 after 6 months storage in open and sealed containers at 8.6% (L) and 13.5% (H) initial seed moisture contents at 30°C/95%RH and 20°C/75%RH.

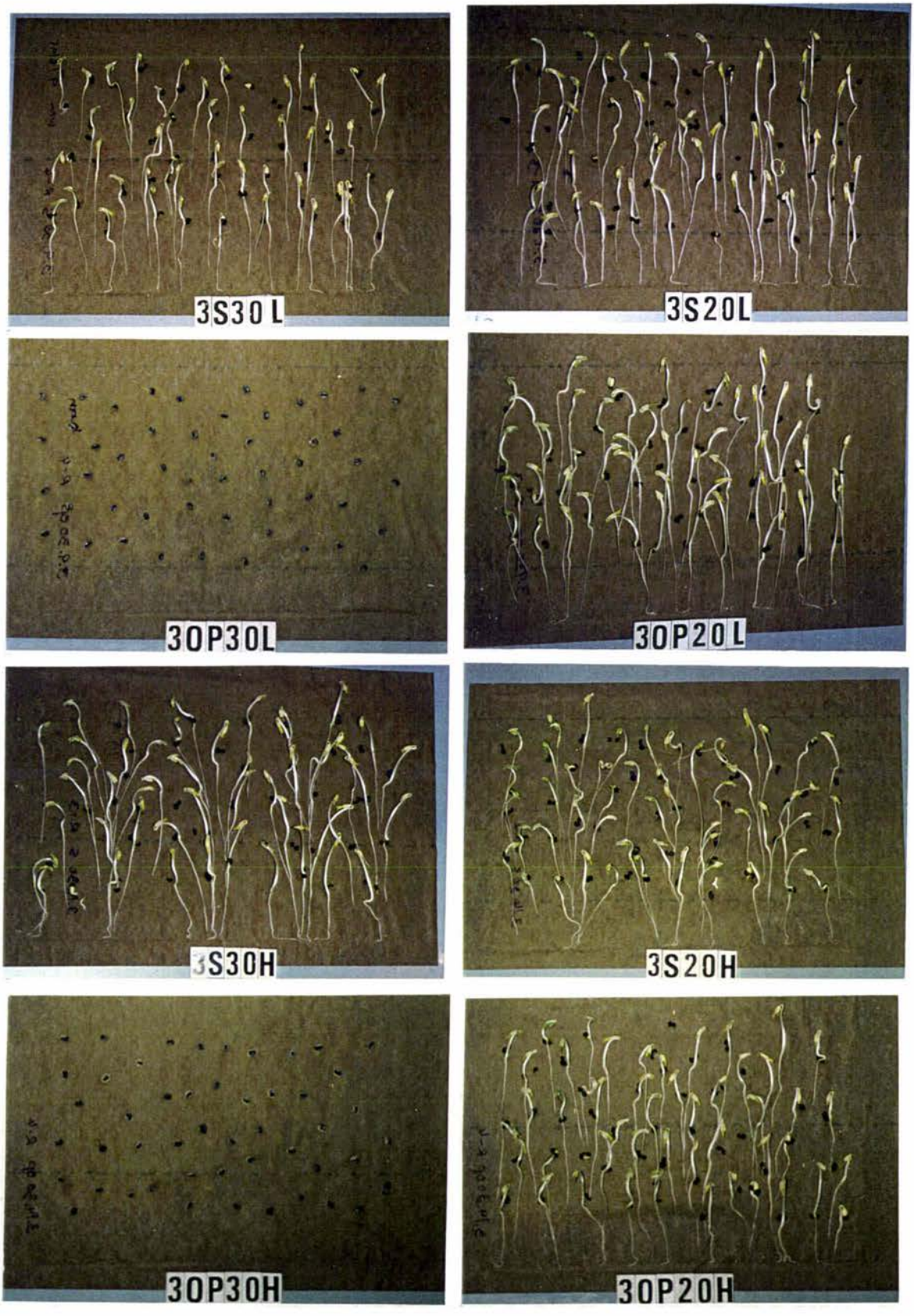

Plate 3 Five day old seedlings of mungbean from seed lot 3 after 6 months storage in open and sealed containers at 8.6% (L) and 13.5% (H) initial seed moisture contents at 30°C/95%RH and 20°C/75%RH.

at 8.6% and 13.5% initial seed moisture contents, showed a sharper decrease in normal germination after 2 months in seedlots 1 and 2, than in seedlot 3. Also at this early storage period, seed stored at an 8.6% initial moisture content lost germination slightly more slowly than seed at 13.5% initial seed moisture contents in lots 1 and 2. In sealed storage, seed stored at 8.6% initial seed moisture content showed virtually no decrease in germination at the end of 8 months storage, while seed stored at 13.5% initial moisture content showed a bigger decrease at 30°C, particularly in seedlot 1 (88% to 76%) and seedlot 2 (94% to 80%) after 8 months storage. Seedlot 3 essentially maintained original germination levels during this time.

At 20°C/75%RH storage, there was no difference in normal germination percentage due to the effect of packaging or initial seed moisture content.

Abnormal seedlings and dead seeds

Percentages of abnormal seedlings and dead seeds were a function of normal seedling level. Changes in percentages and types of abnormal seedlings and dead seeds during storage were also affected by different storage conditions (Table 7).

Under 30°C/95%RH, as stated above, open stored seeds were all dead after 6 months storage. However, sealed stored seeds showed a small increase in abnormal seedling and dead seeds with increasing storage time, particularly in lots 1 and 2. After 8 months storage, lot 1 showed lower total abnormal seedlings than lot 2, but seed of lot 1 died faster than lot 2, particularly in initially wetter stored seeds. There was no difference in the level of damaged seedlings in all three lots but an increase in deformed seedlings occurred, particularly in lots 1 and 2. Decayed abnormal seedlings, particularly in wetter seeds increased in all lots. Such changes were less significant in mungbean seeds stored under 20°C/75%RH, particularly in initially drier seeds stored in sealed containers.

4.2.1.3 Conductivity

Table 7 Percentages of different abnormal seedlings and remainder of three mungbean seedlots after 8 months storage at 30°C/95%RH or 20°C/75%RH

Lot	Initial level (0 month)					Treatment																			
							30°C/95%RH										20°C/75%RH									
							Open *					Sealed					Open					Sealed				
	% ABN				%	% IMC	% ABN				%	% ABN				%	% ABN				%	% ABN				%
	DM	DF	DC	T	REM		DM	DF	DC	T	REM	DM	DF	DC	T	REM	DM	DF	DC	T	REM	DM	DF	DC	T	REM
1	2	1	2	5	7	8.6	0	0	0	0	100	2	5	1	8	11	1	4	2	7	10	1	4	1	6	9
						13.5	0	0	0	0	100	1	4	2	7	17	3	3	4	10	8	2	4	4	10	9
2	2	4	0	6	0	8.6	0	0	0	0	100	3	6	2	11	3	4	7	0	11	1	2	5	1	8	1
						13.5	0	0	0	0	100	2	8	3	13	7	3	7	1	11	1	2	8	1	11	1
3	1	5	0	6	0	8.6	0	0	0	0	100	1	2	0	3	1	1	2	0	3	0	2	1	1	4	1
						13.5	0	0	0	0	100	2	3	3	8	1	2	3	0	5	0	2	2	1	5	0

Note IMC = Initial seed moisture content (%) ABN = Abnormal seedling (%) REM = Remainder (includes hard seed, and dead seed)
* = Data presented are at the end of storage (6 months).
DM = Damaged seedlings (%) include missing root with insufficient secondary roots, broken root, cotyledons and/or terminal bud, and crack hypocotyl.
DF = Deformed seedlings(%) include stunt root, small seedlings, short hypocotyl, no root and short curled hypocotyl.
DC = Decayed seedling (%) include seeding rot and infected by fungi .
T = Total percentage of abnormal seedlings

Although the three mungbean seedlots were different in terms of electro conductivity leakage before storage, levels were low. Subsequently, when seeds were stored at different initial seed moisture contents, packaging, and temperatures/relative humidities for up to 8 months conductivity changed differently. Results are presented in Table 8.

Before storage, seedlot 1 recorded the highest leachate conductivity reading while seedlot 3 had the lowest reading. Nevertheless the variation between lots was only 22.9 to 31.4 μs/cm/g seed. All three seedlots showed an increase in solute leakage with increasing time under all storage conditions although in some treatments this increase was minimal. Seed stored at 30°C/95%RH in open storage gave particularly high conductivity values. In open storage at 30°C/95%RH, seedlots 1 and 2 stored at 8.6% or 13.5% initial seed moisture content showed a sharp increase in conductivity reading at 2 months rising to a peak at 4 months and then declining at 6 months. However, seed of lot 3 showed only a slight increase at 2 months, and thereafter showed a continuing increase through to 6 months storage. In sealed storage, the three seedlots showed a gradual increase in conductivity reading with increasing storage time. However, these increases in all cases were small.

At 20°C/75%RH storage, there was also no great change in electro conductivity reading with increasing storage time or with storage containers in all seedlots. There was, however, a major effect of initial seed moisture content on the leachate conductivity reading in all seedlots in open and sealed storage.

When comparing the results of electro conductivity readings in seed stored in sealed storage at 30°C/95%RH or at 20°C/75%RH, the leachate electro conductivity reading was slightly affected by temperature, being higher at 30°C than at 20°C.

4.2.1.4 Field fungi

Changes in the percentage of field fungal infestation in three different mungbean

Table 8 Effects of different seedlots, initial seed moisture contents of 8.6% and 13.5% , packaging containers (open and sealed containers) on electro conductivity reading (μs/cm/g seed) of mungbean seed after storage at intervals of 0, 2, 4, 6, and 8 months at 20°C/75%RH and 30°C/95% RH. Data presented are means of four replications.

Lot	Initial level (%)	Treatments															
		% ISMC	30°C/95%RH								20°C/75%RH							
			Open			Sealed					Open				Sealed			
			2	4	6	2	4	6	8		2	4	6	8	2	4	6	8
1	31.4	8.6	50.7	112.77	105.4	39.6	36.9	41.3	40.6		37.6	34.2	33.4	35.0	39.5	39.3	40.2	38.5
		13.5	49.9	117.3	101.8	42.8	41.7	49.1	49.4		33.8	32.2	35.3	35.7	37.0	36.3	36.2	35.1
2	26.0	8.6	42.3	120.2	112.7	31.5	30.7	29.9	30.7		27.8	27.2	27.5	27.1	29.2	29.0	28.3	27.5
		13.5	51.5	139.0	122.6	31.1	32.7	32.3	39.7		26.9	27.3	26.2	26.5	27.5	27.1	27.7	27.8
3	22.9	8.6	27.2	90.3	98.5	32.3	34.4	32.9	33.3		24.2	24.3	22.8	23.8	25.4	28.9	30.0	30.2
		13.5	27.3	94.0	99.2	24.5	25.7	26.3	28.0		21.9	23.6	23.0	23.5	21.9	22.3	22.0	22.3

Note: ISMC = Initial seed moisture content (%).
RH = Relative humidity (%)
Time = Storage period (months)

seedlots stored at different initial seed moisture contents (8.6 % and 13.5%) in different types of packaging (open or sealed containers) for up to 8 months at 20°C/75%RH or 30°C/95%RH are presented in Table 9.

At the beginning of the storage period the three seedlots showed different levels of field fungi infection, being highest in seed lot 2 and lowest in seedlot 1. During storage all three seedlots showed a decline in field fungus levels but at different rates depending on initial seed moisture content, packaging type and storage temperature and relative humidity.

Changes in the percentage of field fungi infection, particularly when seeds were stored under more extreme storage conditions (30°C/95%RH) were affected by storage container in all three seedlots. Under open storage seedlots 1 and 2 were totally disinfected from field fungal infection after 2 months. Seedlot 3, however, showed a slower decline with storage time but also showed no field fungi infection after 6 months storage. In sealed storage all three seedlots showed a slower decline with increasing storage time but field fungi were eliminated in lots 1 and 2 at higher initial seed moisture contents after 4 months storage. At 20°C/75%RH, differences in the rate of death of field fungi in different treatments was affected by seedlot. Field fungi in lot 3, for example, were relatively unaffected by storage at 20°C/75%RH, while seeds of lot 2, in particular, showed low levels of field fungi after 8 months storage under these conditions.

Although not a totally consistent relationship, the rate of death of field fungi was generally faster in initially wetter samples, particularly in sealed storage.

The main genus of field fungus found in mungbean during storage was *Alternaria spp* (Table 10). Other genera such as *Fusarium spp.*, *Rhizoctonia spp.*, *Cladosporium spp.* and some unidentified genera also occurred but at very low levels.

There was, however, inconsistency in the level of occurrence of *Alternaria spp.*

Table 9 Effects of different seedlots, initial moisture contents of 8.6% and 13.5% , packaging containers (open and sealed containers) on percentage of field fungi infected seed of mungbean after storage at intervals of 0, 2, 4, 6, and 8 months at 20°C/75%RH and 30°C/95% RH. Data presented are percentages of field fungi infected seeds from 40 seeds (1 seed = 2.5%).

Lot	Initial infected seed (%)	Treatments															
			30°C/95%RH							20°C/75%RH							
		%	Open			Sealed				Open				Sealed			
		0	2	4	6	2	4	6	8	2	4	6	8	2	4	6	8
1	3	8.6	0	0	0	5	3	0	3	5	5	5	3	0	5	3	3
		13.5	0	0	0	5	0	0	0	0	0	8	3	3	0	3	0
2	25	8.6	0	0	0	8	10	3	3	5	10	8	3	18	10	18	5
		13.5	0	0	0	5	0	0	0	18	13	8	8	15	15	5	0
3	13	8.6	5	3	0	15	8	13	8	10	10	10	5	15	13	13	10
		13.5	5	3	0	13	5	3	3	15	13	5	5	15	10	13	10

Note: ISMC = Initial seed moisture content (%).
RH = Relative humidity (%)
Time = Storage period (months)

Table 10 Rate of occurrence of *Alternaria spp.* in mungbean seed during storage at 20°C/75%RH and 30°C/95% RH. Data presented are percentage of fungal colony presented on 40 seeds (1 colony = 2.5%)

Lot	Initial Level (%)	Treatments															
		% IMC	30°C/95%RH								20°C/75%RH							
			Open			Sealed				Open				Sealed			
			2	4	6	2	4	6	8	2	4	6	8	2	4	6	8
1	3	8.6	0	0	0	0	0	0	0	3	3	0	0	0	3	3	0
		13.5	0	0	0	3	0	0	0	0	0	3	3	0	0	0	0
2	18	8.6	0	0	0	5	8	3	3	5	8	8	3	15	10	13	5
		13.5	0	0	0	0	0	0	0	13	10	8	8	15	8	3	0
3	13	8.6	3	0	0	13	8	8	5	5	8	3	0	13	13	8	8
		13.5	0	0	0	13	3	0	0	8	3	5	3	10	8	10	5

Note IMC = Initial seed moisture content. Time = Storage period (months) RH = Relative humidity

- Other genera eg. *Fusarium spp., Rhizoctonia spp., Cladosporium spp.* and unidentified were mostly found only at low levels (1 seed) during storage.

between seedlots and storage times, particularly in seedlots 2 and 3 in both open and sealed storage at 20°C/75%RH and in sealed storage at 30°C, *Alternaria spp.* levels in all samples of seedlot 1 were negligible (Table 10). At 30°C/95%RH in open storage *Alternaria* died in less than 2 months but in sealed storage it was eliminated after 2-4 months in seedlot 1, in initially wetter seed after 2 months in lot 2 and after 8 months in lot 3, In seed stored at 8.6% initial moisture content in sealed storage *Alternaria* survived after 8 months storage in lots 2 and 3 although at low levels. At 20°C/75%RH in open storage *Alternaria* survival varied between lots and storage times. After 8 months open storage *Alternaria* survived in seedlots 2 and 3 stored at 13.5% initial moisture better than in seed stored at 8.6% initial moisture content. In sealed storage at 20°C/75%RH *Alternaria* in seed of lot 1 died earlier than in lots 2 and 3, but by the end of 8 months storage the fungus still survived in lot 3 and in lot 2 at the low initial seed moisture content.

4.2.1.5 Storage fungi

As shown in Table 11, the three seedlots were infected by storage fungi before entering storage at different but generally low levels, being lower in seedlots 1 and 2 (3 % and 5%, respectively) and higher in seedlot 3 (13 %). On subsequent storage with different conditions of packaging, initial seed moisture content, and temperature/ relative humidity for up to 8 months, the level of storage fungi infected seeds of mungbean seedlots altered dramatically.

All seedlots of mungbean were greatly affected by packaging. In particular, seeds in open storage at 30°C/95%RH, showing a rapid increase in the percentage of storage fungi infected seeds to 100% after only 2 months storage, while most seeds in sealed storage showed a marked increase in storage fungi infection with increasing storage time to a peak at 4-6 months, and then showed a decline. Infection levels in lot 3, however, were still high after 6 months storage, particularly in samples initially stored at lower seed moisture content.

Table 11 Effects of different seedlots, initial moisture contents of 8.6% and 13.5% , packaging containers (open and sealed containers) on percentage of storage fungi infected seed of mungbean after storage at intervals of 0, 2, 4, 6, and 8 months at 20°C/75%RH and 30°C/95% RH. Data presented are percentages of storage fungi infected seeds from 40 seeds (1 seed = 2.5%).

Lot	Initial infected level (%)	Treatments															
			30°C/95%RH							20°C/75%RH							
		%	Open			Sealed				Open				Sealed			
		0	2	4	6	2	4	6	8	2	4	6	8	2	4	6	8
1	3	8.6	100	100	100	3	35	25	10	10	58	35	3	3	25	15	8
		13.5	100	100	100	5	60	33	3	8	75	35	3	0	43	30	5
2	5	8.6	100	100	100	3	38	33	15	3	43	35	13	18	20	18	5
		13.5	100	100	100	15	53	45	8	3	55	28	5	5	58	30	10
3	13	8.6	100	100	100	38	63	68	58	38	58	38	20	35	68	70	63
		13.5	100	100	100	28	43	23	18	18	70	23	18	15	45	50	18

Note: ISMC = Initial seed moisture content (%).
RH = Relative humidity (%)
Time = Storage period (months)

At 20°C/75%RH, in most treatments, percentage of storage fungi infected seeds also increased to a peak at 4 months (lots 1 and 2), or at 6 months (lot 3) in sealed storage before declining through to 8 months storage. The percentage of storage fungi at the peak was often higher in seed stored with 13.5% initial moisture content at 20°C. By the end of the storage period, infection levels were still higher than initial levels in all treatments, particularly in drier samples.

The most common storage fungi found in infected mungbean seed were *Penicillium spp.* and *Aspergillus glaucus* in open storage and *Aspergillus glaucus* in sealed storage. Main *Aspergillus* species detected were *Aspergillus glaucus*, *A. flavus*, *A. ochraceus, A. candidus* and *A. niger*. On subsequent storage at 30°C/95%RH (Table 12) and 20°C/75%RH (Table 13) levels of *A. ochraceus* in sealed storage and *A. niger* in all treatments were negligible. *A. flavus* was also present at relatively low levels in most treatments, except for a higher percentage in seedlot 2 in open storage at 30°C/95%RH. *A. glaucus* developed most in all treatments during storage, particularly in open storage at 30°C/95%RH. *A. glaucus, A. ochraceus* and *Penicillium spp.* developed quickly during the first 4 months of storage. However after a further 2 months storage period *Penicillium spp.* became more important, while *Aspergillus* occurrence decreased in all seedlots. In sealed storage at 30°C, the most dominant fungus was again *A.glaucus* although *A. flavus, A.candidus* and *Penicillium spp.* were detected at low levels. Most storage fungi infection levels were either zero or very low after 8 months, except for seedlot 3 where *Aspergillus glaucus* still survived at high levels. By the end of storage, *A. glaucus* was found to have survived better, particularly in lot 3.

At 20°C/75%RH both in open and sealed storage , *A. glaucus* was again the dominant fungus with growth to a high level at 4 months being followed by a decrease after 8 months storage. By the end of storage only seedlot 3 still showed a high level of *A. glaucus,* particularly in drier samples in sealed storage.

Table 12 Rate of occurrence of different storage fungi in mungbean seed during storage at 30°C/95%RH. Data presented are the percentage of storage fungi colonies from 40 seeds.

Lot	Initial infection (%)			Treatments																															
				% IMC	Open															Sealed																
					4							6								4							6							8			
	AG	AF	T		AG	AF	AO	AN	AC	P	T	AG	AF	AO	AN	AC	P	T	AG	AF	AO	AN	AC	P	T	AG	AF	AO	AN	AC	P	T	AG	AF	AO	AN	AC	P	T
1	0	3	3	8.6	83	5	48	0	20	43	209	73	0	10	0	15	80	178	30	10	0	0	3	3	46	18	10	0	0	5	8	41	8	0	0	0	3	0	11
				13.5	68	10	55	0	20	55	208	43	0	20	0	10	83	156	53	8	0	0	13	8	82	23	0	3	0	5	8	39	3	0	0	0	0	0	3
2	0	5	5	8.6	70	13	60	0	8	48	199	40	18	45	0	0	70	173	30	8	0	0	5	5	48	25	3	0	0	5	5	38	5	5	0	0	0	5	15
				13.5	38	25	98	0	0	15	176	8	20	60	0	0	78	166	48	5	0	5	10	0	68	30	0	5	0	10	8	53	5	0	0	0	0	3	8
3	13	0	13	8.6	78	3	18	0	38	73	210	10	8	0	3	15	95	131	63	5	0	0	10	0	78	60	3	0	3	5	3	74	58	3	0	0	0	3	64
				13.5	73	0	33	0	18	90	214	3	3	0	0	10	100	116	35	0	0	0	8	3	46	20	0	0	0	3	3	26	15	0	3	0	0	3	21

Note

IMC = Initial seed moisture content
AG = *Aspergillus glaucus*
AF = *Aspergillus flavus*
AO = *Aspergillus ochraceus*
AN = *Aspergillus niger*
AC = *Aspergillus candidus*
P = *Penicillium spp.*
T = Total

Table 13 Rate of occurrence of different storage fungi in mungbean seed during storage at 20°C/75%RH. Data presented are the percentage of storage fungi colonies from 40 seeds.

Lot	Initial infection (%)			Treatments																									
				% IMC	Open														Sealed											
					4							8							4							8					
	AG	AF	T		AG	AF	AO	AN	AC	P	T	AG	AF	AO	AN	AC	P	T	AG	AF	AO	AN	AC	P	T	AG	AF	AO	AN	AC	P	T
1	0	3	3	8.6	43	3	0	0	10	0	56	0	0	0	0	0	3	3	20	5	0	0	3	3	31	0	0	0	0	0	8	8
				13.5	53	15	0	0	25	0	93	3	0	0	0	0	0	3	30	15	0	3	15	0	63	3	0	0	0	0	3	6
2	0	5	5	8.6	38	5	0	0	13	0	56	8	5	5	8	0	0	26	18	8	0	3	0	0	29	3	3	0	0	0	0	6
				13.5	30	8	0	5	18	0	61	0	3	3	0	0	0	6	45	5	0	3	10	3	66	8	0	3	3	0	3	17
3	13	0	13	8.6	48	5	0	0	13	3	69	18	0	3	0	0	3	24	53	10	0	0	3	5	71	58	0	3	0	0	5	66
				13.5	58	10	0	0	20	3	91	13	0	0	0	0	5	18	28	10	3	3	5	0	49	10	3	3	0	0	3	19

Note

IMC = Initial seed moisture content
AG = *Aspergillus glaucus*
AF = *Aspergillus flavus*
AO = *Aspergillus ochraceus*
AN = *Aspergillus niger*
AC = *Aspergillus candidus*
P = *Penicillium spp.*
T = Total

4.2.2. Performance of peanut seed during storage

From results of initial seed quality evaluation, only peanut seedlot 3 (cv. Spanish white) which had high germination and vigour was selected to study the performance and deterioration rate in this storage experiment. Half the seed in this lot was adjusted in moisture content to 11.5% and the rest was maintained at 6.6% SMC. Seeds at both levels of moisture content were stored under different conditions of packaging (open and sealed storage) and temperatures/ relative humidities of 5°C/85%RH, 20°C/75%RH, 30°C/50%RH and 30°C/95%RH.

4.2.2.1 Seed moisture content (SMC)

The performance of peanut seed in terms of seed moisture content stored under different conditions of packaging (open or sealed), initial seed moisture content and temperature/ relative humidity are presented in Table 14.

The moisture content of peanut seed stored under the same conditions of temperature/relative humidity was greatly affected by packaging. In open storage, for example, seed exchanged moisture with the surrounding air to reach an equilibrium. Under 5°C/85%RH storage conditions seed stored at 6.6% initial moisture content increased to 7.3% after 1 month storage and reached a peak of 8.1% after 4 months storage. However, seed stored at 11.5% initial moisture content lost moisture to 8.0% at 1 month and to 7.2% after 8 months. In 30°C/50%RH and 20°C/75%RH conditions similar changes occurred with seed decreasing from initial seed moisture contents to equilibrium moisture content values of 3.8-4.4% at 30°C and 6.2-6.7% at 20°C. Again these changes occurred mainly within the first month of storage. In open storage at 30°C/95%RH seed showed more extensive increases in seed moisture content to 12.4% in 2 months in seed stored initially at 6.6%, and 12.7% in seed stored at 11.5% initial moisture content. In sealed storage where moisture content of seed was maintained, seeds initially stored at 6.6% or 11.5% initial moisture content under all storage conditions and temperatures showed no change in seed moisture content.

Table 14 Effects of initial seed moisture contents (6.6% and 11.5%), packaging containers (open and sealed) on percentage of seed moisture content of peanut seed after storage at intervals of 0, 1, 2, 4, 6, and 8 months at 5°C/85%RH, 20°C/75%RH, 30°C/50%RH, and open storage at 30°C/95%RH. Data presented are means of four replications.

Treatments																															
%	30°C/95%RH			30°C/50%RH										20°C/75%RH										5°C/85%RH									
IMC	Time	Open		Open					Sealed					Open					Sealed					Open					Sealed				
	0	1	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
6.6	6.6	11.4	12.4	4.4	4.3	4.3	3.9	3.8	6.8	6.6	6.8	6.7	6.7	6.6	6.3	6.7	6.4	6.5	6.8	6.7	6.8	6.8	6.8	7.3	7.5	8.1	7.7	7.1	6.7	6.6	6.8	6.8	6.7
11.5	11.5	11.3	12.7	4.4	4.4	4.3	3.9	3.8	11.5	11.7	11.9	11.9	11.7	6.6	6.2	6.6	6.4	6.4	11.5	11.6	11.9	11.8	11.8	8	7.9	8.3	7.9	7.2	11.5	11.5	11.7	11.5	11.6

Note IMC = Initial seed moisture content (%)
RH = Relative humidity (%)
Time = Storage period (months)

There was no effect of temperature on seed moisture content of seed stored in sealed packets at 5°C, 20°C or 30°C. However, big differences in equilibrium seed moisture content occurred in open packets due to differences in relative humidity (*i.e.* 50%, 75%, 85% and 95%).

4.2.2.2 Germination

Normal seedlings

The normal germination performance of peanut seed stored under different conditions of packaging (open and sealed), initial seed moisture content and temperature/ relative humidity are presented in Table 15 and Plate 4.

Prior to storage, peanut seed had 72% normal germination, and in all storage conditions (except 5°C storage of dry seed) the percentage of normal germination fell, depending on the storage conditions.

At 30°C storage, the germination of peanut seed was greatly affected by relative humidity. In particular, seed in open storage at 95%RH stored at initial moisture contents of 6.6% or 11.5% died in one month while seed stored at 50%RH relative humidity maintained germination better. However, seed stored at different initial moisture contents reacted differently to storage at 50%RH. For example, seed stored initially at 6.6% initial moisture content showed a relatively small loss of germination from an initial 72% to 60% after 8 months, while seed stored at an 11.5% initial moisture content showed a more severe decline to 39% at the end of eight months storage. At 20°C/75%RH storage, a similar effect occurred, although not as severe, with final respective values of 62% and 46%.

In the sealed storage environment at both 30°C and 20°C germination decreased faster in initially wetter samples, seed being dead after two months and six months respectively. Drier samples also lost germination but at a slower rate at 20°C than at

Table 15 Effects of initial seed moisture contents (6.6% and 11.5%), packaging containers (open and sealed) on percentage of normal germination of peanut seed after storage at intervals of 0, 1, 2, 4, 6, and 8 months at 5°C/85%RH, 20°C/75%RH, 30°C/50%RH, and open storage at 30°C/95%RH. Data presented are means of four replications.

Init.		Treatments																													
		30°C/95% RH		30°C/50%RH										20°C/75%RH										5°C/85%RH							
level	%	Open		Open					Sealed					Open					Sealed					Open					Sealed				
(%)	IMC	1*	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
72	6.6	2	0	70	69	66	59	60	70	66	52	43	22	70	75	73	61	62	72	66	66	67	62	75	72	72	70	72	71	75	77	68	67
	11.5	0	0	53	51	39	41	39	17	0	0	0	0	70	61	60	53	46	64	36	9	0	0	76	72	67	62	58	68	56	50	37	31

Note Init. = Initial (normal seedling percentage)

IMC = Initial seed moisture content (%)

RH = Relative humidity (%)

* = Storage period (months)

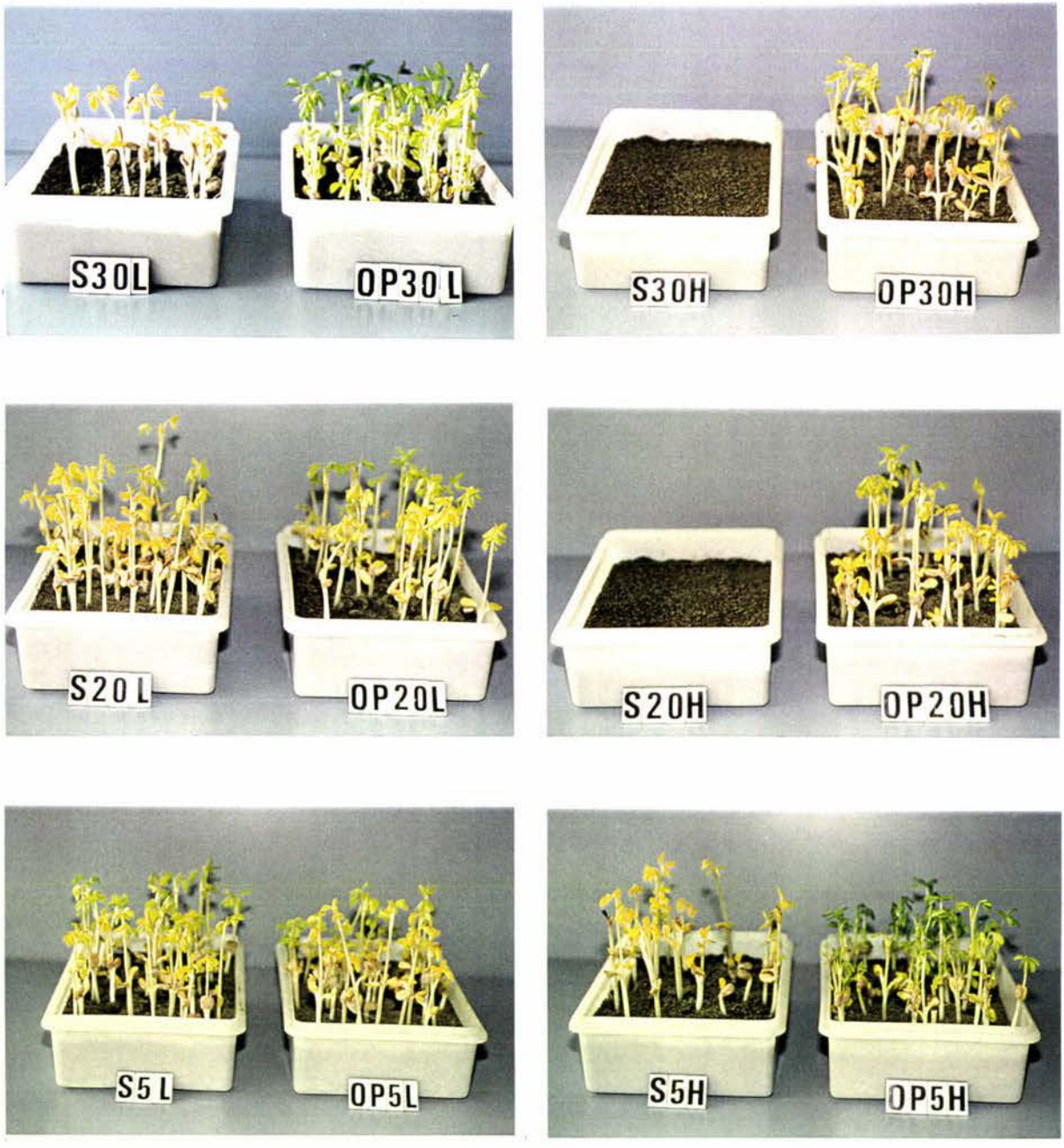

Plate 4 Nine day old seedlings of peanut after 8 months storage in open and sealed containers at 6.6% (L) and 11.5% (H) initial seed moisture contents at 30°C/50%RH, 20°C/75%RH, and 5°C/85%RH.

30°C. Final germination levels of 62% and 22% were recorded after eight months storage respectively.

At 5°C/85%RH, germination of seed in open or sealed containers, particularly at high initial seed moisture contents declined with increasing storage time from 72% at nil storage to 31% - 58% after 8 months. The germination of dry seed stored at 5°C/85%RH however was relatively unaffected.

The germination of both dry and wet seeds in sealed storage decreased at different rates due to the influence of temperature, showing a most rapid decrease in seed stored at 30°C and slowest in seed stored at 5°C. These results clearly show the advantage of storing peanut seed at low initial moisture content in sealed storage and at low temperatures. They also, however, show the deleterious effect of storage of wet peanut seed in sealed storage, particularly at high temperature.

Abnormal seedlings and dead seeds

As peanut seeds lost all normal germination under open storage at 30°C/95%RH after 2 months storage, seeds also lost abnormal germination and dead seeds increased to 100%. After 8 months storage, seeds open stored at 30°C/50%RH, 20°C/75%RH or 5°C/85%RH showed differences in the level of abnormal seedlings and dead seeds relating to level of initial seed moisture content and temperature (Table 16). At 30°C/50%RH abnormal seedling levels were not different from initial levels prior to storage but dead seeds had markedly increased, particularly in seed initially stored at 11.5% SMC. Levels of abnormal seedlings, in particular deformed abnormal seedlings, increased slightly when seeds were initially stored wet at 20°C/75%RH or 5°C/85%RH. Under these storage conditions increases in dead seeds also occurred, particularly in initially stored wetter seed but at a slower rate than at 30°C/50%RH.

All seeds died when stored initially wet (11.5%SMC) in sealed containers at 30°C/50%RH or 20°C/75%RH but at 5°C/85%RH an increased percentage of

Table 16 Percentages of different abnormal seedlings and remainder of a peanut seedlot after 8 months storage at 30°C/50%RH, 20°C/75%RH or 5°C/85%RH

	Treatment																													
	30°C/50%RH										20°C/75%RH										5°C/85%RH									
%	Open					Sealed					Open					Sealed					Open					Sealed				
IMC	% ABN				%	% ABN				%	% ABN				%	% ABN				%	% ABN				%	% ABN				%
	DM	DF	DC	T	R	DM	DF	DC	T	R	DM	DF	DC	T	R	DM	DF	DC	T	R	DM	DF	DC	T	R	DM	DF	DC	T	R
6.6	2	24	1	27	13	4	20	3	27	51	2	21	7	30	8	2	20	7	29	9	1	20	2	23	5	4	20	4	28	5
11.5	2	24	3	29	32	0	0	0	0	100	2	28	3	33	21	0	0	0	0	100	2	26	2	30	12	3	27	6	36	
	Initial level (%)																													
	2	23	2	27	1																									

Note IMC = Initial seed moisture content (%) ABN = Abnormal seedling (%) R = Remainder (dead seed) T = Total
DM = Damaged seedlings (%) include missing root with insufficient secondary roots, broken or deeop cracked roots and/or hypocotyl .
DF = Deformed seedlings(%) include stunt root and/ or stunt shoot, small seedlings and weak terminal bud, short thick hypocotyl without root and/or insufficient secondary root and curled hypocotyl, emerged root but no growth seedlings.
DC = Decayed seedling (%) include seeding rot and severe infected on cotyledons and/or shoot by fungi .

abnormal seedlings (36%) (particularly deformed and decayed abnormalities), and of dead seeds (33%) occurred. Under these conditions there were no differences in abnormal seedling levels in seeds initially stored at 6.6%SMC but dead seed percentage was markedly increased in seeds stored at 30°C.

4.2.2.3 Conductivity

The conductivity test result on peanut seed was 12.9 $\mu S.cm^{-1}.g^{-1}$ seed before storage. However, during storage, conductivity values increased in all treatments (except at 5°C/85%RH in dry seed samples in sealed storage) due to the influence of packaging, initial seed moisture content and temperature/ relative humidity as shown in Table 17.

At 30°C/95%RH, leachate conductivity was greatly affected by relative humidity, increasing rapidly after 1 month while in seed stored at 30°C/50%RH only a small increase occurred with increasing storage time. Conductivity values in all treatments other than 30°C/95%RH were comparatively low, (12.6-16.5 $\mu S.cm^{-1}.g^{-1}$ seed) except in wet seed samples in sealed storage. In this situation, temperature played an important role, resulting in much higher conductivity values at 30°C (54.7$\mu S.cm^{-1}.g^{-1}$ seed) than at 5°C (24.3 $\mu S.cm^{-1}.g^{-1}$ seed). Storage at 20°C gave an intermediate result (42.0 $\mu S.cm^{-1}.g^{-1}$ seed).

4.2.2.4 Field fungi

As shown in Table 18, before storage , approximately 18% of peanut seeds were found to be infected by field fungi (mainly *Fusarium spp.* and *Rhizoctonia spp.*,Table 19). The percentage of field fungally infected seed, however, declined during storage to low levels irrespective of storage conditions, with field fungi being eliminated in 1 month at 30°C/95%RH in open storage and in 8 months in wet seed stored at 30°C in sealed storage. In all other treatments field fungus infection percentage fell from 18% initially to 3-8% after 8 months storage.

Table 17 Effects of initial seed moisture contents (6.6% and 11.5%), packaging containers (open and sealed) on electro conductivity reading (μs/cm/g seed) of peanut seed after storage at intervals of 0, 1, 2, 4, 6, and 8 months at 5°C/85%RH,20°C/75%RH,30°C/50%RH, and open storage at 30°C/95%RH. Data presented are means of four replications.

Init. level	%	30°C/95 %RH		Treatment																											
				30°C/50%RH										20°C/75%RH										5°C/85%RH									
μs/	IMC	Open		Open					Sealed					Open					Sealed					Open					Sealed				
cm/g		1*	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
22.9	6.6	38.0	53.4	19.6	14.3	14.4	14.8	15.8	17.6	15.3	17.5	19.0	24.1	15.4	12.3	12.7	13.5	15.1	15.3	12.3	14.3	13.5	14.9	14.9	11.4	12.9	12.2	14.0	15.1	12.2	13.0	13.2	12.6
	11.5	46.5	65.2	20.8	14.0	17.2	15.7	16.2	30.8	38.6	46.3	48.7	54.7	18.2	13.7	15.7	15.1	16.5	20.2	21.8	31.8	34.8	42.0	15.7	13.1	14.2	13.2	14.6	15.0	14.2	18.0	22.3	24.3

Note Init. = initial (conductivity reading)
IMC = Initial seed moisture content (%)
RH = Relative humidity (%)
* = Storage period (months).

Table 18 Effects of initial seed moisture contents (6.6% and 11.5%), packaging containers (open and sealed) on percentage of field fungi infected seed of peanut seed after storage at intervals of 0, 1, 2, 4, 6, and 8 months at 5°C/85%RH,20°C/75%RH,30°C/50%RH, and open storage at 30°C/95%RH. Data presented are percentages of field fungi infection on 40 seeds.

Init.		Treatments																													
		30°C/95 %RH		30°C/50%RH										20°C/75%RH										5°C/85%RH									
level	IMC	Open		Open					Sealed					Open					Sealed					Open					Sealed				
(%)	(%)	1*	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
18	6.6	0	0	3	5	8	3	3	8	3	3	5	3	5	5	3	3	3	5	5	8	3	5	10	3	8	5	5	13	5	8	10	8
	11.5	0	0	5	3	0	5	3	8	5	3	3	0	8	8	5	10	5	8	3	5	5	5	8	5	5	5	3	8	5	10	5	3

Note Init. = Initial (field fungal infection level)
IMC = Initial seed moisture content (%)
RH = Relative humidity (%)
* = Storage period (months)

Table 19 Rate of occurrence of different field fungi in peanut seed during storage in open and sealed packets at 5°C/85%RH, 20°C/75%RH and 30°C/95%RH, and in open packets at 30°C/95%RH. Data presented are the percentage of colonies of storage fungi found infected on 40 seeds.

Initial field fungal infection (%)					Treatment																											
					Temp./ RH	IMC (%)	Open																Sealed									
							2*					4					8					2					4					8				
F	R	S	O	T			F	R	S	O	T	F	R	S	O	T	F	R	S	O	T	F	R	S	O	T	F	R	S	O	T	F	R	S	O	T
8	8	0	3	19	30°C/	6.6	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
					95%RH	11.5	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
					30°C/	6.6	0	3	0	3	6	0	5	0	3	8	0	3	0	0	3	0	3	0	0	3	0	3	0	0	3	0	0	0	3	3
					50%RH	11.5	0	0	0	3	3	0	0	0	0	0	3	0	0	0	3	0	3	0	3	6	0	3	0	0	3	0	0	0	0	0
					20°C/	6.6	3	0	0	3	6	0	3	0	0	3	0	0	0	3	3	0	3	0	3	6	3	5	0	0	8	3	0	3	3	9
					75%RH	11.5	0	5	0	3	8	0	5	0	0	5	0	5	0	0	5	0	0	0	3	3	0	0	5	0	5	0	0	0	5	5
					5°C/	6.6	0	3	0	0	3	0	8	0	0	8	3	0	0	3	6	0	5	0	0	5	0	3	3	3	9	3	5	0	0	8
					85%RH	11.5	3	0	0	3	6	3	3	0	0	6	0	0	0	3	3	0	5	0	0	5	0	10	0	0	10	0	3	0	0	3

Note Temp = temperature (°C) * = Storage period (months) IMC = Initial seed moisture content (%) F = Fusarium spp.
R = *Rhizoctonia spp.* S = *Sclerotinia spp.* O = Other genera such as *Sclerotium roifsii*, and unidentified fungi.

In addition to *Fusarium spp.* and *Rhizotonia spp.*, there were small amounts of other field fungi such as *Sclerotium rolfsii* and some unidentified fungi present during storage.

4.2.2.5 Storage fungi

Approximately 43% of peanut seeds were initially infected by storage fungi. This level was altered when seeds were stored in different conditions of packaging , initial seed moisture content, and temperature/ relative humidity as shown in Table 20.

At 30°C, the percentage of infected seed in open storage was highly affected by the relative humidity of the surrounding air, showing a rapid increase to 100 percent before 1 month in seeds stored at 6.6% or 11.5% initial moisture contents in 95%RH storage. This effect did not occur in all other storage conditions.

The appearance of storage fungi on peanut seed in both open and sealed storage at 30°C/50%RH, 20°C/75%RH, or 5°C/85%RH varied but tended to decrease with increasing storage time. However, the death of storage fungi was faster in sealed storage at both 30°C and 20°C, but was slower at 5°C. Similar percentages of infected seeds occurred at the end of storage in open storage under all three temperature/ relative humidity storage conditions (13-25%) with seed stored at lower moisture content showing a generally lower level than wetter seeds.

The most dominant storage fungus infecting peanut seed prior to storage was *Aspergillus glaucus*, followed by *A. flavus* and *Penicillium spp.* and a small percentage of *A. niger* (Table 21 and Plate 5). There was great variation in the proportion of each species of fungus during storage with all species decreasing during later storage. *A.glaucus* was the most common storage fungus surviving in all treatments. *A. flavus*, *A. ochraceus*, *A. niger*, and *A. candidus* generally appeared only at low levels in most treatments. However, these fungi increased quickly when seeds were stored at a high relative humidity of 95% at 30°C.

Table 20 Effects of initial seed moisture contents (6.6% and 11.5%), packaging containers (open and sealed) on percentage of storage fungi infected seed of peanut seed after storage at intervals of 0, 1, 2,4, 6, and 8 months at 5°C/85%RH,20°C/75%RH,30°C/50%RH, and open storage at 30°C/95%RH. Data presented are percentage of storage fungi infection on 40 seeds.

Init.		Treatments																													
	%	30°C/95% RH		30°C/50%RH										20°C/75%RH										5°C/85%RH									
level	IMC	Open		Open					Sealed					Open					Sealed					Open					Sealed				
(%)	0	1*	2	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8
43	6.6	100	100	25	30	40	38	18	10	15	23	25	13	30	28	29	23	20	8	23	33	20	10	10	35	28	30	13	18	40	20	35	20
	11.5	100	100	30	20	40	25	25	15	18	30	15	13	13	18	23	25	23	13	15	15	13	8	23	23	43	28	23	30	23	33	40	18

Note Init. = Initial (storage fungal infection level)
IMC = Initial seed moisture content (%)
RH = Relative humidity (%)
* = Storage period (months)

Table 21 Rate of occurrence of different storage fungi in peanut seed during storage in open and sealed packets at 5°C/85%RH, 20°C/75%RH and 30°C/50%RH, and in open packets at 30°C/95%RH. Data presented are the percentage of colonies of storage fungi found infected on 40 seeds.

Initial fungal infection (%)						Treatment																																	
						Temp/ RH	IMC (%)	Open																		Sealed															
								2*							4					8						2					4					8				
AG	AF	AN	P	T				AG	AF	AO	AN	AC	P	T	AG	AF	AN	P	T	AG	AF	AN	P	T	AG	AF	AN	P	T	AG	AF	AN	P	T	AG	AF	AN	P	T
23	10	5	10	48		30°C/	6.6	68	100	100	68	65	0	401	-	-	-	-	-	-	-	-	-		-	-	-	-		-	-	-	-		-	-	-	-	
						95%RH	11.5	28	100	43	55	68	40	334	-	-	-	-	-	-	-	-	-		-	-	-	-		-	-	-	-		-	-	-	-	
						30°C/	6.6	5	13	0	8	8	0	34	25	8	10	10	53	8	5	5	0	18	8	3	0	5	16	13	0	5	5	23	5	5	3	5	18
						50%RH	11.5	5	8	0	3	5	0	21	20	10	8	8	46	15	5	8	0	28	10	3	0	5	18	28	0	0	3	31	8	0	0	5	13
						20°C/	6.6	18	3	0	5	0	5	31	13	13	3	0	29	8	8	5	8	29	10	8	0	5	23	25	5	8	3	41	5	5	3	0	13
						75%RH	11.5	8	8	0	3	0	5	24	18	3	5	0	26	10	13	0	0	23	8	5	0	3	16	13	3	0	0	16	8	0	0	0	8
						5°C/	6.6	13	13	0	5	0	8	39	20	5	0	3	28	3	5	0	8	16	18	13	8	10	49	16	5	3	10	34	5	10	3	3	21
						85%RH	11.5	5	8	0	8	0	3	24	30	10	5	5	50	8	3	13	0	24	8	8	8	3	27	15	8	5	10	38	3	8	5	5	21

Note IMC = Initial seed moisture content
Temp = temperature
* = Storage period (months)
AG = *Aspergillus glaucus*
AF = *Aspergillus flavus*
AO = *Aspergillus ochraceus*
AN = *Aspergillus niger*
AC = *Aspergillus candidus*
P = *Penicillium spp.*
T = Total

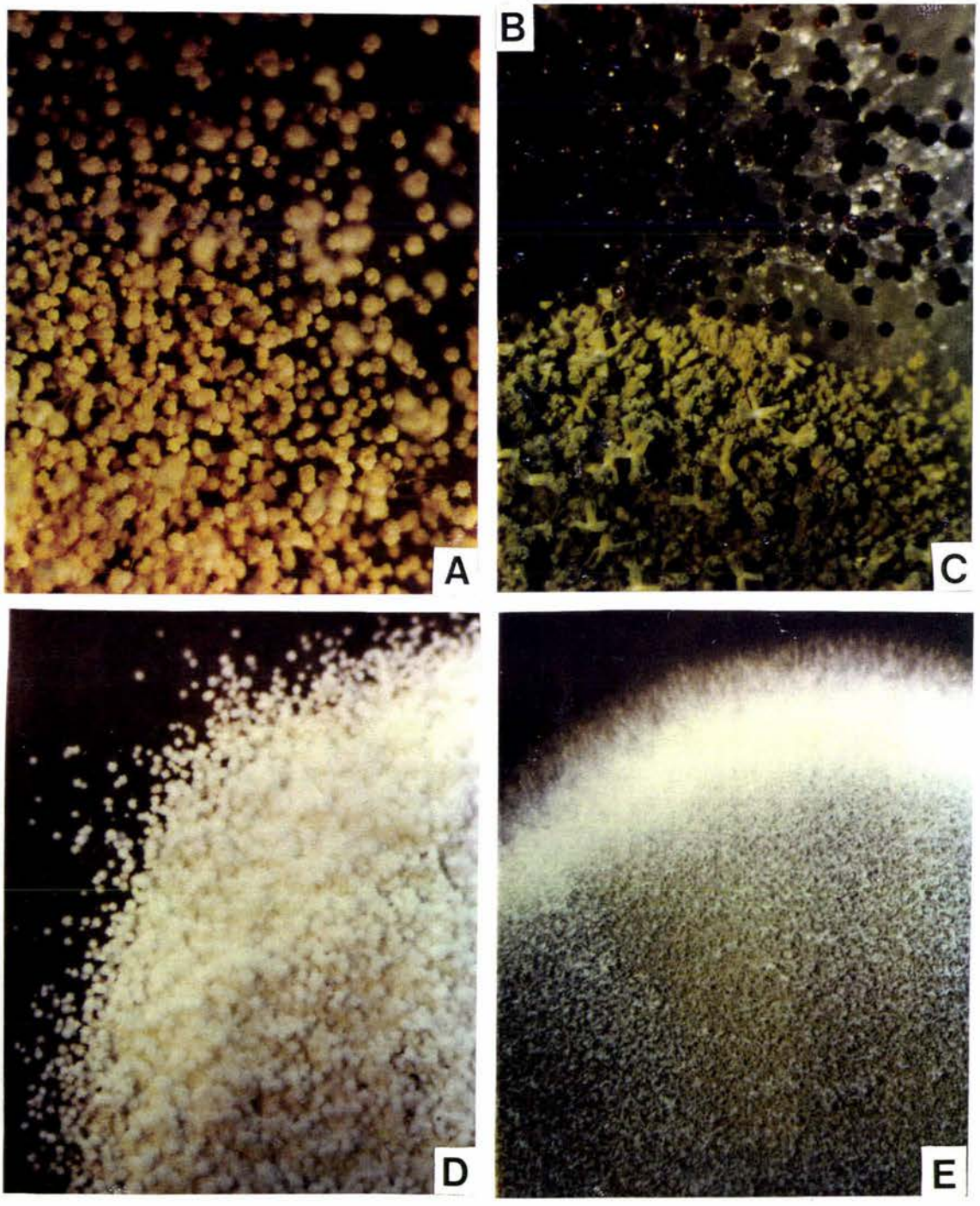

Plate 5. Storage fungi on mungbean and peanut seed. (A) *Aspergillus flavus*, (B) *Aspergillus niger*, (C) *Aspergillus glaucus*, (D) *Aspergillus candidus*, (E) *Penicillium spp.*

CHAPTER 5
DISCUSSION

5.1 INITIAL QUALITY EVALUATION

According to the ISTA rules (ISTA,1993), the purity analysis is used to identify and quantify the composition of the seed lot, *i.e.* pure seed, other seed and inert matter. Pure seeds include intact seeds of all varieties and cultivars of the species being tested, or pieces of seed units larger than one-half their original size (even if immature, undersized, shrivelled, diseased, sprouted or mechanical damaged, and providing the seeds can be identified as of that species). All other matter which is not true seed (pieces of broken or damaged seed units half or less than half the original size, seed without seed coat, and seed with separated cotyledons) are defined as inert matter. Thus, the purity analysis is useful in assessing physical quality of a seed lot.

From purity analysis results in five mungbean seedlots, the presence of physically damaged seeds (*i.e.* impacted or crushed and bruised seeds) in pure seed of lot 1 indicated that seeds of this lot may have been subjected to mechanical abuse during harvesting or threshing while seed moisture content was rather high and before being dried to a lower seed moisture content. The presence of discoloured and/or wrinkled coat seeds and small shrivelled seeds, even in a small percentage, also suggested that seeds of this lot may have been at various stages of maturity at harvest (shiny, green, intact seeds are full mature seeds, discoloured and wrinkled coated seeds are often over-mature and weathered seeds, while small and shrivelled seeds are often immature). These characteristics are likely to have caused seeds in lot 1 to show poorer quality performance than seeds in lots 2 and 3. Uncleaned, slight wrinkled and cracked testas in seed lots 2 and 4 may indicate that these two seedlots were excessively moist prior to harvest. The high level of separated cotyledons and pieces of broken seeds less than half their original seed size in lot 4 indicate that seeds in this lot may have been subjected to impaction forces at a low seed moisture content. Although only a small percentage in visibly damaged seeds were found in the pure seed fraction, internal

damage has also occurred and had further reduced seed quality.

The presence of broken cotyledons in the pure seed and inert matter fractions of all three peanut seedlots, though a small percentage, suggested that these seedlots had undergone mechanical shelling damage. Although this is a common effect resulting from mechanical threshing its low occurrence suggests it was not a serious problem. The presence of skinned seeds and fragile coated and split seeds, particularly in peanut lot 3, might be due to the threshing of relatively dry seeds since Gelmond (1971) and Woodroof (1973) have both reported that peanut seeds threshed at below 7%SMC are readily skinned, split or damaged during shelling.

The variation in seed weight between lots (presented as 1000 seed weight) was likely to relate to seed moisture content and cultivar differences. Among the five mungbean lots, there was no difference in seed weight within the same cultivar (lots 2 and 4) compared at the same seed moisture content level (10%) (lot 2 = 57.3 g and lot 4 = 56.9 g). Lot 1 cv. Chinese and lot 5 also had similar seed weight (56.4 g and 56.0 g) at 10%SMC, respectively). Seed of lot 3 cv. Regur had the highest weight (62.2 g) at the same seed moisture content level. Amongst the three lots of peanut, at the same seed moisture content (10%) lot 2 cv. Virginia had a higher seed weight (754 g) than lot 1 cv. Spanish Red and lot 3 cv. Spanish White (thousand seed weights of 537 g and 490 g, respectively). These differences in seed weight between seed lots are not sufficient to determine quality differences, despite the fact that several reports have shown that an association between seed size and/or seed weight and seed quality (germination and vigour) may be important within a lot (population) but not between lots within a cultivar or between cultivars because of genetic differences. As a result, the same variety may show variation in seed size/weight when produced in different areas and/or years due to nutritional and environmental effects (Delouche, 1980; 1992).

Since mungbean seed can be successfully stored at 12% SMC or less for short-term storage without loss of germination (Chin *et al.*, 1978; Heslehurst *et al.*, 1987), the seed moisture contents of mungbean lots 1, 2, 3 and 4 (10.9-12.9%) are considered 'safe'

for storage without affecting quality in the New Zealand environment. However, the level of seed moisture content of lot 5 (18%) was higher than that considered safe for storage and was likely to be invaded by fungi causing seed death (Harrington, 1972; 1973b).

Seed moisture content level in peanut (oily seeds) is a crucial factor causing reduction in seed quality. Peanut seed should be dried to 6% or below for safe storage (Barton, 1961; Gelmond, 1971; Norden, 1981). Amongst the three peanut seedlots examined, lot 1 and in particular lot 2 had a high moisture content which was too high (8.2%). This may affect seed quality.

Normal germination results provide information about planting value of seedlots under favourable environmental conditions. Poorest normal germination in mungbean lot 5 may be due to the production of abnormal seedlings and dead seeds caused by the influence of high seed moisture content. This was confirmed by the appearance of deformed and mouldy abnormal seedlings and dead seeds. Such poor germination can cause serious problems in future storage. Lower germination in mungbean lot 4 may be due to the presence of damaged seedlings (such as breakage of cotyledons, plumule, hypocotyl and/or radicle), deformed abnormal seedlings and dead seeds. The high levels in damaged abnormal seedlings confirmed that mungbean lot 4 had been subjected to mechanical damage. Seed lot 1 which was also presumed to have been subjected to mechanical damage, showed lower levels of damaged abnormal seedlings but a higher dead seed percentage suggested that lot 1 may contain higher levels of seed so severely damaged that they are dead, but also low levels of slightly injured seeds. Small and invisibly damaged seeds may not cause immediate loss in normal germination, but may become increasingly important during ageing (Moore, 1972). Thus, lower levels of normal germination present after accelerated ageing in mungbean lot 4 might have been at the expense of nonvisibly damaged seeds which could not withstand the high humidity and temperature conditions used and produced more abnormal seedlings as a result.

Poor germination both before and after accelerated ageing in all three peanut seedlots (particularly lot 2) indicated that many seeds had already begun to deteriorate and lose both germination and vigour. Such loss might be due to the fact that these peanut seedlots have been stored for a period of time at a relatively high seed moisture content (in particular lots 1 and 2) prior to testing. However, seeds of lot 3, which contained lower moisture, still maintained germination at acceptable commercial levels in Thailand (70%).

Differences in seed vigour of mungbean and peanut lots were confirmed by the conductivity test. The conductivity testing detects loss of membrane integrity determined by measurement of electrolyte leakage from imbibed seeds into soak water, the higher readings being interpreted as lower vigour (Pandey, 1992, Hampton, 1994b). Leachate conductivity generally increases with decreasing seed viability and increasing visual damage or cracks in the testa (Oliveira *et al.*, 1984). The higher conductivity reading and therefore lower seed vigour in mungbean lot 5 and peanut lot 2 in this study may well be associated with increased cell membrane permeability of deteriorated or membrane damaged seeds and dead seeds as seeds aged at high moisture levels. Such deteriorated or membrane damaged seeds may allow so rapid water uptake that the repair and reorganisation processes of cell membranes can not cope with the influx of water. As a result the cell contents leak into the surrounding medium at a high level (Powell and Matthews, 1979; Oliveira *et al.*, 1984; Crowe *et al.*, 1989). Higher levels of conductivity in mungbean lot 1 than in lot 4, suggest that slightly damaged or invisibly damaged seeds in lot 4 may have had more ability to repair and reorganise cell membranes than lot 1 which contained high levels of externally damaged seeds. However, leachate conductivity reading is not only a function of the level of seed coat cracking or permeability and the ability to repair or reorganise membrane within the seed, but also varies with the amount of organic and inorganic ions in the cells (Coolbear, 1994). Cultivar differences may also partly involve differences in the level of leakage due to variable permeability characteristics of the seed coat (Verma and Ram, 1987).

Detection of field fungi revealed that *Alternaria spp.* was the most common field fungus infecting mungbean seeds, particularly lot 2. *Fusarium spp.* and to a lesser extent *Rhizoctonia spp.* were the main field fungi infecting peanut seedlots. *Alternaria,* as well infection by other field fungi is often quiescent in seeds, because fungal mycelium and spores may contaminate or infect dry seeds without showing any visual symptoms and may also not affect seed germination in storage since these fungi require high seed moisture contents in equilibrium with a relative humidity of more than 90% to grow (Christensen, 1972; Rotem, 1994)). In some cases these field fungi, especially *Fusarium,* may cause discolouration and shrivelling of seeds, weakening or death of the embryo and produce toxic compounds (Christensen, 1972). These field fungi, however, are much more likely to cause seed decay, damping-off and seedling rot when seeds are germinated (Porter *et al.*, 1984), and, as a result, may be primary causes of poor germination and vigour in the field.

Poor quality in peanut seeds, particularly in lots 1 and 2 and in mungbean lot 5, is more likely to be associated with infection by storage fungi, since these seedlots were infected with high levels of *Aspergillus spp.* (eg. *A. glaucus*, *A. flavus* and *A. niger)* and *Penicillium spp.* Seeds with a moisture content above 12% are likely to be invaded by storage fungi (Harrington (1972), while in peanut seeds containing 6-10% SMC *A. glaucus,* and *A. flavus* and *Penicillium* may all be readily encountered (Christensen and Sauer, 1982). Christensen and Kaufmann (1965) and Christensen (1973) have also stated that storage fungi have the ability to grow in dry seeds whose moisture contents are in equilibrium with relative humidities of 70-90% and that they may discolour and kill the embryos and cause spoilage and heating of stored seeds. This deterioration is usually accompanied by biochemical changes eg. increases in free fatty acids, and mycotoxin accumulation.

5.2 THE PERFORMANCE OF MUNGBEAN AND PEANUT SEEDS DURING STORAGE.

The initial quality of the three mungbean seedlots used in this study varied in

terms of both seed germination and vigour. Seedlot 1 was poorest in terms of these quality parameters and, although lots 2 and 3 had similar levels of germination, lot 2 had lower vigour than lot 3. Unfortunately limited seed supplies of peanut only allowed one seed lot to be used for the storage experiment. This lot was also of low quality (germination 72%).

Immediately prior to storage, seed from each lot was adjusted to one of two different moisture contents. The lowest moisture content was 6.6% for peanut and 8.6% for mungbean which are considered to be 'safe' storage moisture contents for these species (Justice and Bass, 1978). The higher moisture levels, chosen to provide correspondingly 'unsafe' storage moisture contents, were 13.5% for mungbean and 11.5% for peanut. The time taken to obtain these various seed moisture levels varied between species and depended on initial pre-adjustment seed moisture content and whether seed of each species was required to lose or gain moisture from its initial seed moisture content (11.5% for mungbean and 6.6% for peanut) to obtain the required adjusted level. For example the time taken to increase seed moisture content at 5°C, 100%RH from 11.5 to 13.5% in mungbean was 65-68 hours compared to 5 days to raise seed moisture content of peanut from 6.6-11.5%. This agrees with similar work by Herath *et al.* (1981) on mungbean seed and Loeffler *et al.* (1988) on soybean seed moisture equilibration at 20°C. The slower rate of moisture absorption in peanut may relate to both its relative size and high oil composition (Barton, 1961; Bennett-Lartey, 1991). Since ageing of seed, particularly legumes, occurs at high relative humidity the time involved in moisture adjustment may have caused some loss of seed quality. This possibility is supported by work on peanut seed by Manda (1993), who found that 4 of 5 seedlots of peanut lost 10-14% germination during the process of increasing seed moisture content from 6.3% up to 14% at 20°C. The adjustment of seed moisture content for this experiment, however, was conducted at 5°C which may have reduced this effect. However, initial seed quality is also an important factor affecting subsequent quality after increasing seed moisture content. This was particularly true in peanut which had an initial germination of only 72%. The method used to decrease seed moisture content to a lower moisture content by placing seed in a desiccator over dry

silica gel at room temperature, is, however, unlikely to have had any adverse effect on seed germination and vigour. A similar technique was used by Zhang and Tao (1988) in a study on drying of beans, maize, rice, cucumber and cabbage seeds. Despite their suggestion that low moisture content may induce hard seed in legume seed, this was not a problem in either species in the present study.

During storage seed moisture equilibrium was markedly affected by the type of packaging container used, to a lesser extent by temperature, and to a greater extent by relative humidity in open storage treatments. Since controlled storage conditions were used (30°C/95%RH, 20°C/75%RH for mungbean, and 30°C/95%RH, 30°C/50%RH, 20°C/75%RH and 5°C/85%RH for peanut), changes in equilibrium moisture content in each treatment was primarily a response to relative humidity/ seed moisture content exchange in open storage, although relatively minor effects also occurred at different temperatures. In particular, due to the hygroscopicity of seed, open storage conditions allowed seed moisture content to exchange and come to equilibrium with the relative humidity of the surrounding air, the higher the relative humidity in the storage environment, the higher the seed moisture content obtained. This situation has been previously observed by other workers (eg. Harrington, 1972; Delouche *et al.*, 1973; Justice and Bass, 1978). In the present study, for example, mungbean seed open stored in muslin cloth bags increased from initial seed moisture contents (8.6% and 13.5%) to equilibrium moisture contents at 75% RH and 95%RH after 8 months of 11.3-12.7% and 24.6-26.6% respectively. A similar effect occurred in peanut, although equilibrium moisture content was reached at a lower level. This can be explained by differences in seed composition since seeds containing higher levels of protein are more hydrophilic, and seeds containing high levels of lipids are hydrophobic. Seeds containing carbohydrates are intermediate in this respect (Barton, 1961, Justice and Bass, 1978). This is well illustrated in this study since peanut seed contains up to 45-50% oil and 25% protein (Kadam *et al.*, 1989), with a resultant low water absorbing capacity compared to mungbean which contains approximately 1.5% oil and 25-28% protein (Lawn and Ahn, 1985).

The well known hysteresis effect was particularly obvious in mungbean stored at 20°C/75%RH where the equilibrium seed moisture content after absorbing moisture in initially dry seeds was approximately 0.5% lower than the equilibrium seed moisture content obtained in samples which desorbed moisture from an initially high level. This situation agrees with similar findings by Justice and Bass (1978) and Pixton (1982) who showed that at the same relative humidity the equilibrium moisture content of seeds may not be the same, desorbing seeds containing higher moisture content than absorbing seeds. Somewhat surprisingly, however, this hysteresis was not found in peanut seed, a situation also reported by Pixton (1982) who also showed that there was no measurable effect of hysteresis when peanut seed was stored at 15°C, 25°C or 35°C. In this study, the lower equilibrium seed moisture content in seeds of mungbean cv. Berken than in other lots was probably due to slightly lower initial seed moisture content.

The relatively constant levels of seed moisture content retained in mungbean and peanut seed lots stored in aluminium foil packets confirmed that the sealed packaging system used was impermeable to seed moisture or relative humidity exchange. Since the level of seed moisture content (and/or relative humidity) is the prime factor that can affect seed quality and storability or deterioration rate of seed (Barton, 1961; Delouche *et al.*, 1973; Justice and Bass, 1978), sealed packaging has become an important option in preventing seed moisture content change during storage, particularly in humid environments where relative humidity is high (Arvier, 1983).

Seed deterioration rate can be affected by relative humidity, which controls seed moisture content, but also by temperature, which affects the rate of deteriorative biochemical processes in seeds (Harrington, 1972). Certainly, losses in germination of seed progress very rapidly under high relative humidity and temperature storage conditions (Harrington, 1973a), a situation which was well illustrated in mungbean and peanut seed in open storage at 30°C/95%RH (Tables 6 and 15) in this study. Under such conditions mungbean and peanut seeds in all treatments gained very high moisture contents (15-18%SMC in mungbean and above 12%SMC in peanut after 2 months

storage), and this was followed by rapid loss in normal germination and an increase in dead seed percentage. The higher ability of mungbean lot 3 (cv. Regur) to retain seed germination during early storage may well be due to its higher initial quality. Compared to peanut, however, mungbean seedcoat structure is relatively less permeable to moisture absorption (Kadam *et al.*, 1989). The rapid loss of seed viability due to the effects of high relative humidity and temperature is also found in many other crop species. As examples, beans and sweetcorn seeds have been shown to lose total germination after 92 days when stored at 25°C and 79%RH (Lin, 1988); soybean seeds retain viability for only 4 months when stored at 25°C and 85%RH (AVRDC, 1990) and peanut seeds lose viability rapidly when stored at 30°C and 90 %RH (Aung, 1991).

Storage conditions involving high relative humidity and temperature also caused problems during an 8 months storage period with rapid seed deterioration being due to increased activity of storage fungi. As stated by Harrington (1968), seed deteriorative changes in seed during storage occur due to alteration in seed biochemistry. Such changes include reduction in extractable nucleic acids (Ketring, 1971), lipid peroxidation which is mediated by free radicals and lipoxygenase (Wilson and McDonald, 1986), reduction in fatty acids (Singh and Yadav, 1987; Nautiyal and Zala, 1991) and esterase activity (Aung and McDonald (1995). Therefore, seeds of many crop species should not be stored under such adverse conditions if retention of germination is important.

The results of this storage experiment clearly support the suggestion by Barton (1961) that seed deterioration decreases as relative humidity or seed moisture content decreases. In open storage of peanut seed at 30°C/50%RH initially stored at either 6.6% or 11.5% SMC seed moisture content fell to around 4% after 1 month and maintained germination better than at 30°C/95%RH, though some loss in seed viability with increasing storage period, particularly in seeds initially stored at 11.5% moisture content still occurred. The initial quality of seed prior to storage, the exposure of seed to high humidity during moisture adjustment, and the high initial seed moisture content before reaching a low seed moisture content equilibrium with the storage humidity might all

be responsible for this decline in seed viability. It is also likely that temperature effects are involved. Aung (1991) has reported that high quality peanut seeds (90% germination) stored at 50%RH or below, or with a moisture content about 6% or less could be kept at 30°C for 30 weeks without significant loss of germination, although lower quality seeds (54%) deteriorated more rapidly than higher quality seeds (70%).

Similarly, mungbean seeds open stored at a moisture content of 12% in all lots at 20°C/75%RH also maintained germination (82-83% in lot 1, 88-89% in lot 2 and 95-97% in lot 3) after 8 months. Similar results occurred in peanut. When peanut seeds stored at 20°C/75%RH or 5°C/85%RH equilibrium moisture content equilibrated at around 6.5% and 7-8% after 1 month storage, according to the level of relative humidity, the germination of seed was ranked according to temperature level. This suggests that seed moisture content (or relative humidity) and temperature may reinforce and compensate each other in their effect on seed longevity as stated by Delouche *et al.* (1973).

Moisture-proof packaging can be used to prevent the deleterious effect of high humidity and prolong seed germination and vigour provided only dry seeds (6-12%SMC for starchy seeds and 4-9% of oily seeds) are placed in the containers (Harrington, 1973*b*). Clearly, seed deterioration rate in sealed storage depends directly on the moisture content of seed entering storage and the temperature of the storage environment. For example, in sealed storage where seed moisture content did not change from initial levels (Table 5), all mungbean seedlots maintained germination satisfactorily during 8 months storage, particularly in seeds stored at 8.6% SMC. However, the effect of different initial seed moisture contents on seed viability appeared at 8 months in all lots, particularly in lots 1 and 2, suggesting that in mungbean seed germination can only be maintained at 13.5%SMC for 6 months at 30°C, depending on initial quality. Certainly, differences in seed longevity between seedlots varies with genotype, pre- storage history and storage environment (Ellis and Roberts, 1980). Seeds of mungbean lot 1 performed poorer than lots 2 and 3 due to their lower initial quality (Table 1) which was presumed to be preconditioned by them being subjected to

improperly mechanical damage prior to storage.

Feistritzer and Kelly (1978) have recommended that for safe storage, legume seed should be dried to less than 12%., while Chin *et al.* (1978) reported that mungbean seeds can be stored at 8-12%SMC in sealed containers for 3 months at 26°C without reduction in germination. Similarly, Singh and Yadav (1987) found that mungbean seeds with an initial moisture content of 12% could maintain germination for 2 months in airtight conditions at 27°C without deteriorative change but afterwards declined, while seeds with 16%SMC showed complete loss of germination after 6 months.

The effect of initial seed moisture content on seed viability during storage was also obvious in peanut seed at all temperatures. For example, at 30°C, seed stored at a high initial moisture content in aluminium foil packets lost germination more rapidly (0% germination after 2 months) than seeds stored at a lower moisture content (66% germination after 2 months). A similar rapid loss in seed viability due to high seed moisture content has also been reported by Manda (1993) who showed that in peanut seed stored at 10%SMC in sealed aluminium foil packets, germination declined from 80% to about 20% after 12 weeks storage at 25°C, while seeds with an initial 14%SMC lost all germination after 12 weeks storage. At high temperature (35°C), seeds stored at 10% or 14% were dead after 8 weeks and 4 weeks, respectively. Thus, the most critical consideration is the seed moisture content at the time seed is placed in sealed storage (Harrington, 1972, 1973*b*). Oil seeds above 9% moisture content should not be stored in sealed containers since such moisture levels cause seed to die more rapidly than identical seeds stored in open containers (Harrington, 1972, 1973*b*). Similarly, Navarro *et al.* (1989) recommended that to maintain 90% germination in shelled peanut seeds(cv. Hanoch and Congo) for 6 months, seeds should be stored at about 8 %SMC at 15°C or at 7%SMC for higher temperature storage (26°C). If the seed moisture content is too high, seed respiration and microbial activity (fungal and bacterial) will be so high that the seed will increase in moisture content (water being one of the products of respiration) and respiration will continue until the seed is dead (Harrington, 1973*b*). Respiration uses up oxygen in sealed containers. The anaerobic conditions

which result encourage the production of ethanol and aldehydes, both of which are toxic to seeds and cause loss of seed germinability during storage (Hendry, 1993). These volatile compounds may be evolved and accumulate in sealed storage containers of even dry seeds but the rate increases with increasing relative humidity and temperature, particularly in terms of aldehydes (Zhang *et al.*, 1994). Under these conditions fermentation of wet seeds becomes a problem leading to rapid seed deterioration (Christensen, 1972).

Although, storage temperature plays a secondary role in affecting seed deterioration after seed moisture content, it is nevertheless important, particularly in peanut seed. Temperature effects may reflect differences in the heat tolerance of the two species used, oil seeds being more sensitive to high temperature (Hill, 1995*b*). Harrington (1972) has, however, shown that deterioration rate is slower when seed is stored under cooler conditions than in a warmer conditions. This was confirmed by peanut seed storage results in this study. For example, at 5°C, peanut seed could be stored at 6.6% moisture content in sealed storage for 8 months without loss in germination, while at 20°C seed lost germination at a slower rate (at 8 months) than seed stored at 30°C. The deterioration of peanut seed stored at 6.6% at 20°C was much more rapid than that reported by Norden (1981), who maintained the viability of Spanish type peanut (91% germination) with a moisture content of about 6 % for 3 years when seed was stored below 20°C; and than the results reported by Navarro *et al.* (1989) that peanut seed could be maintained at 90% germination for 6 months at 7%SMC/26°C. The low prestorage quality of the peanut seedlot used in the present study was likely to have been a major contributing factor to such storage performance differences.

Storage conditions can also affect the percentage and type of seedling abnormalities, particularly since high seed moisture content and/or temperature can induce deformed abnormal seedlings with stubby roots, weak seedlings with no development of root hairs or plumule (Neergaard, 1977). In this study seeds which become abnormal during storage under adverse conditions may then quickly become

dead. This was shown in peanut seeds initially stored wet in both open and sealed containers, particularly at 30°C. Except in open stored seeds at 30°C/95%RH where seeds died rapidly, higher levels of dead seeds in mungbean lot 1 after 8 months storage, particularly when sealed stored at 30°C may be due to increases in the death of abnormal seedlings during the extent of the storage period.

Changes in seed vigour in mungbean and peanut seed during storage under different conditions were measured by the conductivity test, a method which has been recommended for detecting vigour differences in large seeded legumes (Hampton *et al.*, 1992; Hampton and Tekrony, 1995). Conductivity readings confirmed that seed vigour was influenced by storage conditions, since they showed a negative relationship with seed germination results, especially in seeds stored under adverse conditions of high relative humidity or seed moisture content and temperature. The longer seeds were stored under such conditions the higher the electrical conductivity of the seed leachate. For example, in open storage at 30°C/95%RH, both mungbean and peanut seeds stored at either low or high initial moisture contents gave very high conductivity readings after 2 months storage (or 1 month in peanut). This indicated that loss in seed vigour accompanies and often precedes loss of viability. Gorecki *et al.* (1985) also found this phenomenon in stored pea seeds under similar conditions. Such losses have been shown to be due to deteriorative changes including membrane damage, and loss of enzyme activity (Pearce and Abdel Samad, 1980; Wilson and McDonald, 1986). Although such changes were not examined in this study, their effects have been investigated by many researchers, eg. in soybean (Priestley and Leopold, 1979), peanut (Pearce and Abdel Samad, 1980), rice (Ghosh *et al.*, 1981), and maize (Basavarajappa *et al.*, 1991).

Damage to cell membranes is suggested as the major cause of seed deterioration and loss of membrane impermeability leading to increased electrolyte leakage during imbibition (Powell and Matthews, 1977; Parrish and Leopold, 1978; Ghosh *et al.*, 1981; Wilson and McDonald, 1986). One probable factor involved in membrane damage is the effect of membrane lipid peroxidation by free radicals, leading to loss of phospholipid from membranes and loss of ultrastructure (Villiers, 1980; Powell and

Matthews, 1981, and Hendry, 1993). Increased susceptibility to hydrolytic enzyme attack (eg. esterases which cause degradation of membrane bound lipids and release fatty acids) may also be involved (Villiers, 1973). In peanut seeds, storage lipids might be important because they occur abundantly (47% lipid comprising 45% oleic and 30% linoleic). These unsaturated fatty acids are particularly susceptible to peroxidation (Wilson and McDonald, 1986). In many cases the subsequent overgrowth of fungi on seeds and their metabolites can also break down plasma membranes (by their withdrawal from the cell wall and rupture) and mitochondrial tissue (Harman and Granett, 1972; Anderson and Baker, 1983). Mite attack may also cause severe damage to cell membranes. These aspects of seed deterioration caused by adverse storage conditions are often so severe that the repair and reorganisation mechanisms of cell membranes during early imbibition cannot cope with the uncontrolled and rapid influx of water. As a result solutes 'leak' from the cells (Crowe *et al.* 1989).

The importance of optimum seed moisture content for safe storage in sealed containers, is very obvious in this experiment, and particularly in peanut. Storage temperature greatly affects the influence of seed moisture content on loss of membrane integrity and seed vigour. This was revealed by wetter seeds of mungbean and particularly of peanut stored in sealed containers at 30°C which gave higher electrical conductivity readings than seeds stored at a lower temperature. At such high temperatures increased metabolism or respiration continues at a high rate until limited by inactivation of enzymes (Justice and Bass, 1978), which result in a cumulative and more deteriorative effect.

The levels of field and storage fungi detected before storage varied between seedlots. Field fungi such as *Alternaria*, *Fusarium* and *Rhizoctonia* died gradually during storage mainly due to their inability to tolerate low seed moisture content. Since field fungi require free water or a relative humidity above 95% for their growth, they are not likely to reinvade in storage (Christensen, 1973). The absence of field fungi in both mungbean and peanut stored in open storage at 30°C 95%RH may also be due to overgrowth by storage fungi which increase rapidly under these conditions. In cases

where drier seeds were stored in sealed containers at different temperatures field fungi survived better than in wetter seeds, particularly at lower temperature. This agrees with similar studies by Dorworth and Christensen (1968) in soybean, Nandi *et al.* (1982) in sesame, mustard and linseed, and Kabeere (1995) in maize. In all of these crops field fungi (eg. *Alternaria,* or *Fusarium*) decreased with increasing storage time and at a more rapid rate in wetter seeds and higher storage temperatures.

It has been shown that seeds already invaded by storage fungi and therefore partly deteriorated, which are then kept under conditions that permit fungi to increase (such as high moisture content or relative humidity and temperature), will be invaded further and lose quality faster than uninfected seeds (Christensen, 1972). Under 30°C 95%RH storage condition, storage fungal mycelium and sporulation developed rapidly on stored mungbean and peanut seeds following an increase in seed moisture content. Changes in the dominant fungal species with time may be related to their different water requirements for growth. In this study, *Aspergillus glaucus* was the pioneer species to infect seeds and became increasingly more active with increasing moisture content in storage. At higher seed moisture contents (above 17% in mungbean or 12% in peanut at 2 months storage) *A. candidus*, *A. ochraceus, A. flavus,* and *Penicillium,* as well as *A. niger* in peanut seed, became more dominant and *A. glaucus* levels decreased, particularly in initially wetter seeds. This competition between fungal species, suggests that the growth of storage fungi requires a moisture content in equilibrium with relative humidities of about 65-90%, the minimum relative humidity range that allows different storage fungi to develop being, 70-73% for *Aspergillus glaucus*, 80% for *A. candidus*, and *A. ochraceus*, 85% for *A. flavus* and 80-90% for *Penicillium* (Christensen, 1973; and Neergaard, 1977) and above 90% for *A. Niger* (Semeniuk, 1954).

The presence of seed caking and musty odour in this study indicated fungal spoilage which was in an advance state of seed deterioration and too late to remedy as previously suggested by Twiddy (1994).

Changes in the level of storage fungal infection in mungbean in open storage at

lower temperature and relative humidity (20°C 75%RH), was also related to seed moisture content (approx. 12% SMC). Similar changes in mungbean seed sealed stored at 13.5%SMC, however, may be because moisture contents permit storage fungi, particularly *A. glaucus*, to grow and produce metabolic water which slightly raises seed moisture content before fungal death occurs because of lack of oxygen.

The absence of storage fungi in sealed stored peanut seeds at all temperatures may also be associated with infection by bacteria (particularly anaerobic acid-forming bacteria) which become dominant after seeds deteriorate under low oxygen conditions (Christensen, 1972). The development of a fungal-bacterial antagonism effect was perhaps supported by increasing numbers of bacterial colonies on PDA plates in wetter peanut seed samples stored in sealed containers (range of 23% - 38% at 5°C-30°C). Schenck and Stotzky (1975) have stated that volatile compounds evolved from imbibing seeds can also cause increased bacterial growth.

CONCLUSION

The initial quality of different mungbean and peanut seedlots assessed by using a number of laboratory test methods such as purity, seed moisture content, germination, accelerated ageing, electrical conductivity, and seed health can be used to identify the cause of quality differences, or pre-storage history, and information about likely storage potential. Purity results can be used to give information on physical characteristics of seedlots. Germination test results not only describe germination potential under favourable conditions but also can be used to detect causes of poor seed quality from the appearance of different types of abnormal seedlings (ISTA, 1993). Vigour tests (accelerated ageing and electrical conductivity tests) are also helpful in determining potential field performance and storability, particularly in high germination seedlots which may have low vigour and do not resist adverse conditions both in the field and in storage. Conductivity can also indicate damage to cell membranes by natural deterioration and mechanical damage. The level of fungal infection on seeds in seed health tests not only indicates seed health status of seed but also indicate likely prestorage damage and the effects of seed moisture content.

The results of storage studies show performance differences between different seed lots of mungbean and peanut, which relate to the initial quality of seed entering storage or pre-storage history when stored under various storage conditions. Seed lots with high initial quality (germination and vigour) deteriorate slower than seed lots with lower initial quality even under adverse storage conditions.

Different performance of seedlots occurred under different storage conditions. Under storage at 30°C and 95%RH which is frequently experienced in Thailand, both mungbean and peanut deteriorated rapidly and seriously, losing germination and vigour after 2 months in mungbean or 1 month in peanut. Such a storage environment causes seeds increase in moisture content to a high level with a concomitant increase in infection by storage fungi, seed death and electrolyte leakage. Under the same

conditions, the use of moisture-proof packaging can satisfactorily prolong seed germination and vigour. Mungbean seed stored wet at 13.5%SMC can survive in sealed storage for at least 6 months even in lower quality lots, but peanut seeds dry stored at 6.6%SMC do not survive such condition.

Thus, for short-term storage of high quality mungbean seed lots, lower temperature conditions might not be necessary if seed moisture content can be kept at or below 13.5% SMC. However, peanut seed may require low temperature storage (eg. 20°C or 5°C) for acceptable storage performance. Despite these species differences, results clearly show that seeds which have been deteriorated prior to storage, will continue to deteriorate in storage even under cool, dry conditions eg. 20°C/75%RH seed with moisture content 6.6%SMC either stored in open or sealed storage can allow peanut seed to be stored without loss in viability for 6 months. At lower temperatures (5°C/85%RH) peanut seed quality can be maintained for an ever longer period particularly since under these conditions there is comparatively low invasion by storage fungi.

Under ambient conditions in Thailand, sealed storage at low seed moisture content is likely to be the most cost-effective option for mungbean seed storage before planting in the following growing season, but peanut seed may need to be stored in sealed containers at lower temperatures in an air-condition room (20°C). Such a recommendation reflects the relative temperature sensitivity and moisture tolerance differences of the species concerned.

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